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CONTENTS

Viability of Tuberclle Bacilli. JOSEPH E. POTTINGER.....	111
A New and Simplified Medium for Pasteurella Tularensis and other Delicate Organisms. B. W. RHAMY.....	121
A Study of Pathogen-Selective Cultures in Relation to Vaccine Therapy. FRED BOERNER AND MYER SOLIS-COHEN.....	125
A Study of O and H Agglutinins in Typhoid and Endemic Typhus Fever. HARDY A. KEMP.....	133
Exploring Death. H. J. CORPER.....	145
Lymphomatous Compression of the Spinal Cord. FREDERICK H. LAMB.....	155
"Sensitivity" to Sulphydryl. STANLEY P. REIMANN.....	167
Editorial.....	171
News and Notices.....	175
Book Reviews.....	177

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VIABILITY OF TUBERCLE BACILLI*

EFFECT OF MECHANICAL SHAKING AND CHEMICALS USED IN CONCENTRATION TECHNIC

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Inoculation of untreated materials into test animals has been the rule in the past, because of possible ill effects of chemicals and prolonged mechanical treatment on the viability of tubercle bacilli. It has been considered better to risk the loss of one or more animals by contaminating organisms than to face possible failure through lowering of virulence of the tubercle bacilli by previous treatment.

The main reason for chemical treatment is the elimination of contaminating organisms particularly for culture work and guinea-pig inoculation. By employing the mechanical shaker a further desirable result is attained in that the tubercle bacilli are scattered uniformly through the material to be examined, so that all portions of equal amount contain approximately the same number of organisms.

In the investigation of sputum, there is a growing tendency to use material saved for several days. The justification for such procedure is mathematically certain, but the disadvantage lies in two directions: the increase in contaminators and the possible ill effect of the aging of the specimen on the viability of the tubercle bacilli contained therein. The collection of a three-day specimen of sputum for microscopic examination has been routine practice in our institution for twenty-two years, from all patients in whom tubercle bacilli have previously not been found and also in those carrying rare tubercle bacilli. This method described and advocated elsewhere¹ applies to about one-half of all specimens ex-

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

amined. No evidence has been noted of the disappearance of tubercle bacilli from these specimens, as some writers fear, but doubt has existed as to their viability.

A large literature is available dealing with virulence and pathogenicity of tubercle bacilli for guinea-pigs but as most of the work has been carried out with pure cultures, it is not reasonable to assume that conclusions, derived from that method of investigation are tenable, when working with tubercle bacilli from body secretions, from exudates, or from lesions in other inflammatory conditions. The environment is markedly different. Moreover, the dosage of tubercle bacilli in most experiments has been too high for comparison with dosage given to many of our animals, in practice. Working with an accurate counting method, it was shown² that the dilution-flotation-pieic acid procedure would detect tubercle bacilli in a ten-minute search in sputum, when present to the number of only 70 to 400 per cubic centimeter of material, depending on the nature of the specimen. The use of the guinea-pig is materially restricted thereby.

Having been engaged for some time in checking this procedure by guinea-pig inoculation, it seemed highly desirable to determine the viability of the tubercle bacilli under the mechanical and chemical influences employed in practice. A further departure from accepted procedure was necessary in order to secure smooth films for counting, in that all dilutions were made with distilled water instead of with salt solution. The crystallization of salt disturbs seriously the uniformity of the film. The experiments to be described are an attempt to determine what effect, if any, these various treatments have upon the viability of the tubercle bacillus.

EXPERIMENTAL CONDITIONS

Six fresh purulent morning sputums were collected from a selected group of patients running a subacute course, in order that grouping of tubercle bacilli might be reduced to a minimum, and greater accuracy of count secured. Each sputum was diluted with equal parts of 0.6 per cent NaCl, shaken for thirty minutes in a strong shaker, and divided into three fractions.

Weighed portions of the first fractions, 1 cc. more or less, were diluted at least 1:30 with distilled water and shaken for twenty minutes, from which the number of tubercle bacilli per cubic centimeter was determined. The emulsions for counting were diluted further with distilled water and shaken ten minutes, in order to secure the desired number of tubercle bacilli per cubic centimeter for dosage. Other weighed portions, 1 cc. more or less, of the first fractions were treated with 0.5 per cent NaOH at 37°C. for one to three hours, diluted and shaken similarly for dosage.

The second fractions were set at 37°C. for fifteen to forty hours and shaken for ten minutes. Weighed portions, 1 cc. more or less, were treated with equal parts of 0.5 per cent NaOH for one to two hours, and other weighed portions with 3 per cent NaOH for twenty-five to thirty-five minutes and diluted exactly as in the first fractions for dosage.

The third fractions were allowed to stand at room temperature ranging from 15° to 28°C. for five to nine days and shaken for ten minutes. Weighed portions were treated with 0.5 per cent NaOH for one to four hours and 3 per cent NaOH for thirty to thirty-five minutes, diluted and shaken as above for dosage.

THE COUNTING METHOD

Precision slides, tested by micrometer, were selected so that a variation in thickness at the four corners and center did not exceed $\frac{1}{16}$ mm. These were placed on a plate glass platform which had been leveled by spirit level over a drying oven. The sputum diluted at least 1:30 with distilled water was shaken by mechanical shaker for thirty minutes. Exactly 1 cc. of this homogenized dilution was placed on the slide and allowed to dry. (If perfectly level the film dries symmetrically with respect to both axes of the slide in about fifteen minutes.) The slides were stained as usual, decolorized with 5 per cent H₂SO₄, for one minute and immersed in water five minutes. The slides were covered with 10 per cent sodium sulphite to which had been added one-fifth volume of 95 per cent alcohol, until thoroughly bleached (five to ten minutes). The slides were immersed in water for five minutes so that all sulphite was removed. If the pink color returned another application of sulphite was made, followed by washing. Pieric acid, 1 per cent, was used as a counterstain. The slide was placed on top of and perpendicular to a slide held by the mechanical stage. A small amount of cedar oil between the slides kept them in place. Every bacillus was counted from side to side 2 cm. from each end, using a limiting frame in the ocular.

The slide was 75,000 micra in length and the frame 89.5 micra in width. Therefore, $75000/89.5 = 838$, the factor determining the number of tubercle bacilli per cubic centimeter of diluted sputum from the average of the counts made.

It was found that unless a purulent sputum was diluted at least 1:30 the film would be too thick, causing some of the tubercle bacilli to be understained and likely to be overlooked in counting. The desirable dilution for the count yielded from 100 to 400 tubercle bacilli, from side to side, averaging about one to four bacilli per field. If the number exceeded this, a further dilution with shaking was made for the final count. Under these conditions maximum penetration of stain was attained in 95 per cent of all bacilli. The number of pale bacilli were counted and if they exceeded 5 per cent, the preparation was discarded. This standard was not difficult to attain, and failure of the first effort occurred but in one of the six specimens.

TABLE 1
COUNTS OF TUBERCLE BACILLI BY THE PRECISION SLIDE METHOD

SPUTUM	NUMBER OF TUBERCLE BACILLI COUNTED				MEAN	PROBABLE ERROR OF THE MEAN
	A	B	C	D		
1	93	116	111	166	121	<i>per cent</i> ± 8.6
2	156	179	191		175	± 3.9
3	250	303	274	239	266	± 3.5
4	447	485			466	± 2.8
5	435	452			443	± 3.3
6	140	146			143	± 1.4

A few counts were made on the second and third fractions. The results were usually within the probable error of the counting method, and no evidence of growth was found. The count of the second fraction, which had been set at 37°C. for fifteen to forty hours, was usually less than that obtained on the first fraction, and some of the tubercle bacilli found took the stain poorly as has been repeatedly pointed out by other workers. The number of pale bacilli exceeded 5 per cent and were found only by proceeding very slowly.

If the first two counts did not vary more than 15 per cent of the lesser, the result was considered satisfactory; if greater than 15 per cent, additional counts were made. The probable error in a 15 per cent variation is ± 5.1 per cent.

Table 1 gives the detailed counts with the probable errors computed. With the exception of the counts for sputum 1, the probable errors are about the same as in careful leukocyte counting. The time required to make a count across the slide averaged about ten minutes.

ANIMAL EXPERIMENTS

Table 2 gives the detailed record of treatment and dosage of tubercle bacilli, and the results, at autopsy, in guinea-pigs.

All guinea-pigs, with the exception of one, showed widespread tuberculosis usually with caseation in one or more of the mesenteric nodes and spleen, less frequently in the liver, pancreas, bronchial glands, and lungs. Those inoculated intraperitoneally, showed involvement frequently in bronchial lymphatic nodes and

TABLE 2
SHOWING RESULTS OF GUINEA-PIG INOCULATION WITH SPUTUM

Shaking mechanically with and without previous treatment with sodium hydrate, 0.5 per cent and 3 per cent. Guinea pigs killed from 56 to 61 days after inoculation.

METHOD OF INJECTING GUINEA-PIGS	DOSAGE OF BACILLI	FRACTION 1 FRESH SPUTUM				FRACTION 2 SPUTUM AT 37°C.				FRACTION 3 SPUTUM AT 12°-28°C.			
		Treated with		Result	0.5 per cent	Result	Treated with NaOH		Result	0.5 per cent	Result	Treated with NaOH	
		H ₂ O	NaOH		hours		hours	min.		days	hours	min.	Result
Left axilla.....	200	++							9	1	++		
Left axilla.....	300	++	3	++	24				6	4	++	30	+
Left axilla.....	200	++	1	++	17	2	++	25	5	2	++	35	++
Intraperitoneal.	100		2	++	40	2	++	30	5	1	++		
Intraperitoneal.	100		1	++	23	2	++	25	5	2	++		
Intraperitoneal.	50	++	3	++	15	1	++	25	5	2	++	30	++

+= tuberculosis of small degree.

++= wide spread tuberculosis.

lung. In the one exception yielding an unsatisfactory result, the inoculum treated with 3 per cent NaOH was injected in the axilla. The organisms were recovered from the inoculation site and axillary nodes but all other organs appeared normal and no tubercle bacilli could be demonstrated in them.

Tubercle bacilli were searched for in preparations made from the caseous material or crushed organs after thorough grinding first without, then with 0.5 per cent NaOH. In some organs in

which bacilli were not found or were rare, the digested material was treated by the dilution-flotation procedure. Because of the high protein content it was necessary to carry out the dilution feature much farther than is necessary in sputum. Approximately 1 gram of digested organ was diluted to 500 cc. with distilled water before shaking with 0.5 cc. of xylol. Erlenmyer flasks were used for the purpose. Usually suspected lesions were small, weighing but a few milligrams, and were removed,

TABLE 3
EXAMINATION OF ORGANS OF INJECTED GUINEA-PIGS

A comparison of direct smear and dilution-flotation method with records of numbers of tubercle bacilli found in the organs of animals and number of minutes spent in search.

ORGAN	METHOD				ORGAN	METHOD				
	Direct smear		Dilution- Flotation			Direct smear		Dilution- Flotation		
	Bacilli	Min- utes	Bacilli	Min- utes		Bacilli	Min- utes	Bacilli	Min- utes	
Spleen	0	20	10	5	Mesenteric nodes	40	5	50	5	
	2	20	45	5		1	20	10	5	
	0	20	15	5		6	5	83	5	
	3	5	63	5		3	10	23	5	
	3	10	13	5		0	10	2	15	
	4	5	255	5		3	1	75	10	
	0	5	3	5		0	10	10	2	
Liver	1	20	8	5	Axillary nodes	3	5	46	5	
	0	10	1	10						
Lung	0	20	1	10	Injected area					
Bronchial nodes	0	5	3	5		14	5	154	5	
	8	5	24	5						

ground and digested for thirty minutes, then treated in a test tube with 5 to 10 cc. of water and 4 to 6 drops of xylol for the dilution-flotation procedure.

Table 3 gives a few comparisons of results obtained by the direct smear and dilution-flotation method, showing the marked superiority of the latter.

DISCUSSION

Adequate control against spontaneous infection in these animals is found in the fact that concurrently with the experimental

work forty-three guinea-pigs of the same stock were routinely inoculated as a check on the dilution-flotation procedure: in thirty-two instances both tests were negative; in four, both were positive; in five, the dilution-flotation was positive and guinea-pig negative; and in two, the dilution-flotation was negative and guinea-pig positive.

The experimental conditions under which this work was carried on presents two features wholly at variance with the customary procedure: first, the use of a strong shaking machine instead of grinding in a mortar to secure uniform distribution of tubercle bacilli; second, the substitution of distilled water for saline solution as a dilutent, for technical reasons. Willis⁴ recent work in showing the gradual loss of pathogenicity of dry tubercle bacilli ground in a ball mill suggests that grinding as usually employed may not be wholly free from effect on the viability of the tubercle bacilli. The grinding of dry bacilli would no doubt result in much greater damage than when carried out under the usual moist conditions.

Strong evidence has recently been given³ that tubercle bacilli in sputum are injured by contact with 3 per cent NaOH for thirty minutes. On the other hand much stronger solutions have been employed as digestants, after which viability was demonstrated by guinea-pig inoculation. One wonders in the latter instance whether the homogenization was adequate to assure actual contact with the chemical. The action of 0.5 per cent NaOH on the tubercle bacillus seems not to have been determined. It is a complete solvent of pus and cells composing organs, though it acts more slowly than stronger solutions. As a digestant it seems to accomplish all that can be accomplished by stronger solutions, and its effect on the viability of the tubercle bacillus, if any, should be less. It does not kill all contaminants in sputum but does inhibit their influence in determining mortality in guinea-pigs.

Of a total of 110 animals inoculated from material of diverse origin, after treatment by 0.5 per cent NaOH, only two died from subcutaneous and two from intraperitoneal inoculation within three weeks. These four deaths resulted from inoculation of sputum which had been in contact with 0.5 per cent NaOH for

one to three hours. The maximal time of contact with the digestant without producing injury to the tubercle bacilli is not known, but is being investigated. To date successful inoculation has followed contact in individual instances for forty, thirty-six, thirty, twenty-two and sixteen hours. Such results suggest the possibility that prolonged contact may eliminate the mortality due to contaminants without doing injury to the tubercle bacilli.

SUMMARY AND CONCLUSIONS

Six different sputums were homogenized by mechanical shaker and divided into three fractions: the first fractions were used fresh; the second stood at 37°C. for fifteen to forty hours; and the third stood at room temperature 15° to 28°C. for five to nine days. Portions of the three fractions were treated with 0.5 per cent NaOH for a period of one to four hours. Portions of the second and third fractions were treated with 3 per cent NaOH for twenty-five to thirty-five minutes. A dosage of fifty to 300 tubercle bacilli following each of these treatments resulted in widespread tuberculosis in twenty-five of a total of twenty-six guinea pigs in fifty-six to sixty-one days. One guinea pig, inoculated with 300 tubercle bacilli from a fraction which stood at air temperature for six days, followed by treatment with 3 per cent NaOH for thirty-five minutes, did not develop widespread disease.

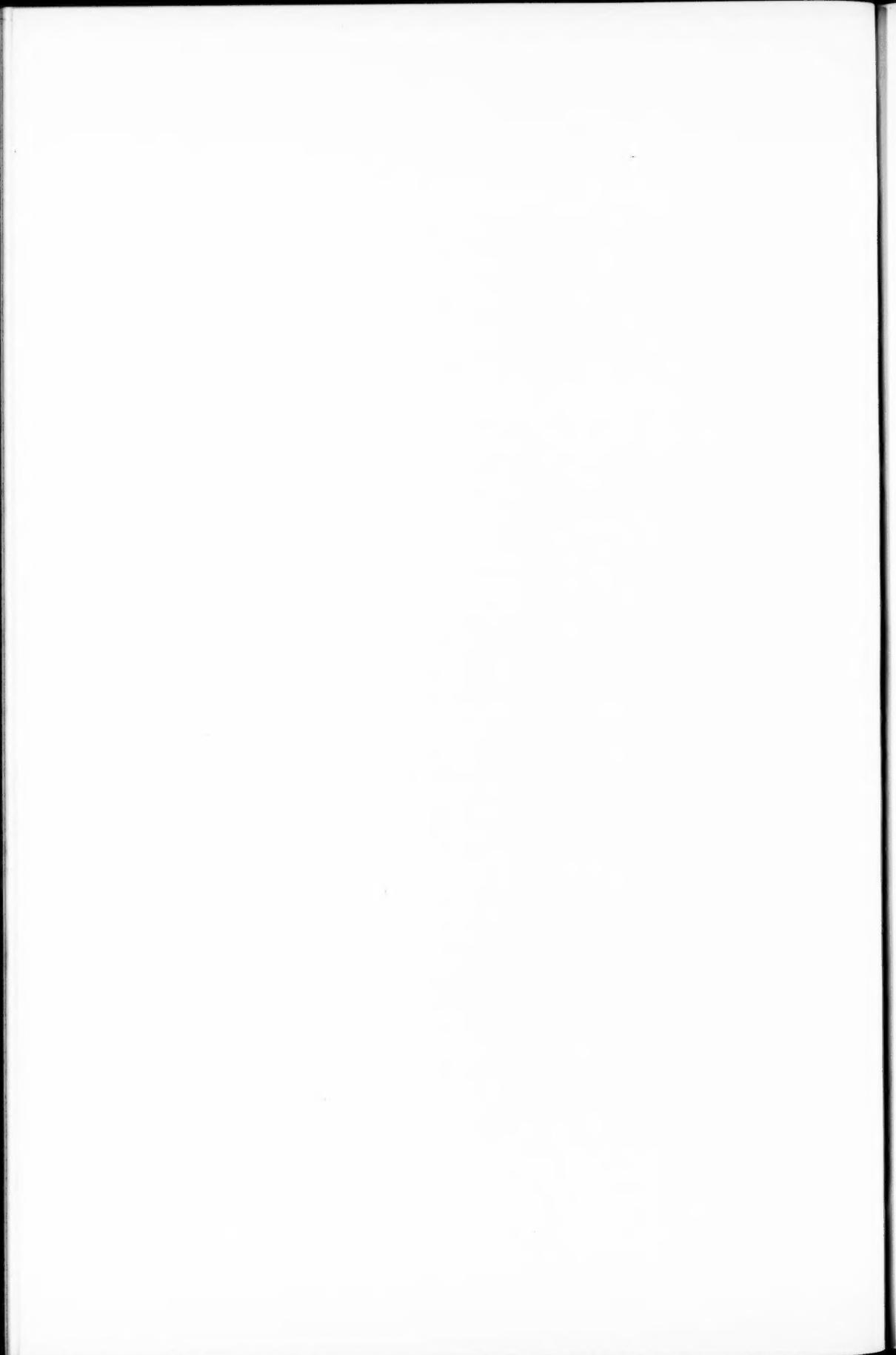
Dilution with distilled water, followed by mechanical shaking for one hour, apparently caused no injury to tubercle bacilli found in fresh sputum.

Similar treatment of fresh, autolyzed (37°C. for fifteen to forty hours) and old specimens (six to nine days) of sputum, after previous digestion of the specimens with 0.5 per cent NaOH for one to four hours, apparently caused no injury to the tubercle bacilli, but injury is probable when 3 per cent NaOH is used as a digestant, for twenty-five to thirty-five minutes.

REFERENCES

- (1) POTTERER, F. M.: Clinical Tuberculosis, Chapter XX, St. Louis, 1922, C. V. Mosby Co., Vol. 1, pp. 550.

- (2) POTTENGER, J. E.: The demonstration of rare tubercle bacilli in sputum by the dilution-flotation method in conjunction with picric acid. Am. Rev. Tuberc., **24**: 583-595. 1931.
- (3) STADNICHENKO, A., AND SWEANY, H. C.: A comparison of culture and animal inoculation of sputum in the diagnosis of tuberculosis. Am. Jour. Clin. Path., **1**: 303-313. 1931.
- (4) WILLIS, H. S.: The effect of mechanical grinding on tubercle bacilli. Am. Rev. Tuberc., **25**: 224-230. 1932.



A NEW AND SIMPLIFIED MEDIUM FOR PASTEURELLA TULARENSIS AND OTHER DELICATE ORGANISMS

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Since the discovery of *Pasteurella tularensis* (*Bacterium tularensis*) by McCoy and Chapin³ the widespread interest in tularemia which followed the accurate work of Francis^{1,2} and of Simpson^{5,6} has made it imperative that clinical laboratories be equipped to make agglutination tests for this disease commonly known as "rabbit fever."

In practice, the technical difficulties attending the preparation of suitable fresh media and keeping alive cultures of *P. tularensis* to furnish sufficient antigen supply are such that the small laboratory finds difficulty in keeping prepared for the infrequent calls for this test. The organism was cultivated by McCoy and Chapin on Dorsett egg medium, on which a scant transparent growth occurred in five days, by Wherry and Lamb⁸ on coagulated egg yolk, by Francis on horse or rabbit serum-glucose agar enriched by a bit of healthy rabbit spleen, and on Loeffler's blood serum coagulated at 20°C. On all of these media the growth appeared in four to six days as minute transparent droplets resembling the white of egg. Francis has since adopted fresh blood-glucose-cystine agar as the most satisfactory medium for *P. tularensis*. This contains 1 per cent peptone, 1.5 per cent agar, 0.5 per cent sodium chloride and is adjusted to a pH of 7.3. When needed there is added to the stock agar 0.1 per cent amino-acid in the form of cystine and 1 per cent glucose; this is heated in an Arnold sterilizer sufficiently long to melt the agar, and to dissolve and sterilize the cystine and glucose. The mixture is then cooled to 40 to 45°C. when 5.0 to 8.0 per cent of defibrinated rabbit blood is added, after which it is tubed, slanted and incu-

bated to test sterility. Precaution must be taken against overheating with loss of the bright red color. Cystine is not readily soluble so that in the Francis medium some of it remains undissolved. Shaw⁴ attempted complete solution by placing the mixture in a water bath at 100°C. or in an autoclave at 15 pounds pressure for fifteen minutes. Transplants were made on this medium every two months from stock cultures kept in the ice box.

In my experience there is considerable hazard in preparing blood-glucose-cystine agar owing to technical difficulties, particularly in regard to the collection, manipulation and addition of sterile rabbit blood subsequent to sterilization of the medium, and difficulty of dissolving cystine, and finally the uncertainties as to the sterility of the media, for contaminations will show up later to spoil the cultures, that were not brought out by the test incubation. Thus, cultures died out or were contaminated on sample medium made by myself and by two biological supply houses.

Since there is no available source from which antigen can be obtained, an effort was made to overcome these difficulties. Following the suggestion of Spray⁷ on the use of heated blood derivatives for cultivation of hemoglobinophilic organisms, the idea presented itself of using dehydrated hemoglobin instead of fresh defibrinated rabbit blood. This would make the medium easier to prepare and safer to use since all ingredients could be sterilized. Thus, having a stock dehydrated cystine agar and stock dehydrated hemoglobin any laboratory could make fresh medium for *P. tularensis* as needed.

Collaborating with the Difco Laboratories, Detroit, Michigan such a fool proof medium has been evolved and has so far proved entirely satisfactory. This medium is not offered as being superior to blood-glucose-cystine agar, but rather a dehydrated, prepared stock medium that can be autoclaved and with which any laboratory can routinely prepare such amounts of medium as meet their requirements. This medium gives a luxuriant growth of *P. tularensis* within three to four days, of cream gray color, furnishing ample material for making antigen. The new medium is made by mixing Spray's Bacto hemoglobin in equal parts, with

Bacto cystine heart agar, which latter is essentially a modified Huntoon hormone agar.

Formula of dehydrated "Bacto Cystine Heart Agar" (Difco)

Beef heart Infusion from.....	500 gm.
Bacto peptone.....	10 gm.
Bacto dextrose.....	10 gm.
Sodium chloride.....	5 gm.
l-cystine.....	1 gm.
Bacto agar.....	15 gm.

Reaction: pH 6.8. (The reaction may range between 6.8 and 7.3 but in my hands 6.8 gave the most luxuriant growth.)

Both the Bacto cystine heart agar and Bacto hemoglobin can be obtained in dehydrated form as a stock supply.

Method for preparing 500 cc.

(A) "Double strength agar"—Dissolve, by boiling 28 grams Bacto cystine heart agar in 250 cc. distilled water. Sterilize for twenty minutes at 15 pounds pressure (250°F.). The sterile agar will be dark in color (chocolate agar).

(B) Dissolve 5 grams Bacto hemoglobin in 250 cc. distilled water and strain through gauze to remove any large undissolved particles. Sterilize twenty minutes at 15 pounds pressure.

(C) Cool both of the above sterile solutions (A and B) to 50 to 60 C. and mix. Dispense in sterile test tubes or other containers. Use strictly aseptic conditions. Incubate to test sterility.

Besides its special value as *P. tularensis* medium* and on account of its very high nutritional qualities, which make it sensitive, this medium is also suggested for routine use as a general purpose medium, especially for cultivation of the various difficulty grown organisms such as the gonococcus, *Hemophilus influenzae* and *pertussis*, the meningococcus, pneumococcus and streptococcus. The luxuriant growth of most organisms makes it valuable for autogenous vaccines or antigens of any kind. It is particularly useful in growing pneumococci and streptococci, and

* At my request Dr. Walter M. Simpson tested the dehydrated cystine heart agar against his own medium. He reported that one sample with pH 7.3 gave fairly satisfactory growth of *P. tularensis* but that the second sample pH 6.8 yielded a very luxuriant growth. He concluded that this formula makes a most satisfactory medium for the cultivation of *P. tularensis*.

in studying their hemoglobinophilic properties, since it gives broad zones of decolorization, changing from dark chocolate to eight brown.

REFERENCES

- (1) FRANCIS, EDWARD: Cultivation of *Bacterium tularensis* on media new to this organism. U. S. Hyg. Lab. Bull. No. 130, p. 44. March 1922.
- (2) FRANCIS, EDWARD: Symptoms, diagnosis and pathology of tularemia. Jour. Am. Med. Assn., **91**: 1155-1161. 1928.
- (3) MCCOY, G. W., AND CHAPIN, C. W.: Further observations on a plague-like disease of rodents with a preliminary note on the causative agent, *Bacterium tularensis*. Jour. Inf. Dis., **10**: 61-72. 1912.
- (4) SHAW, F. W.: Sterilization of cystine culture medium under steam pressure. Jour. Lab. and Clin. Med., **16**: 294-295. 1930.
- (5) SIMPSON, W. M.: Tularemia (Francis' Disease). Ann. Int. Med., **1**: 1007-1059. 1928.
- (6) SIMPSON, W. M.: Recent developments in tularemia (Francis' Disease). Jour. Lab. and Clin. Med., **15**: 311-322. 1930.
- (7) SPRAY, R. S.: Heated blood and blood derivatives for cultivation of the hemoglobinophilic organisms. Jour. Lab. and Clin. Med., **16**: 166-168. 1930.
- (8) WHERRY, W. B., AND LAMB, B. H.: Infection of man with *Bacterium tularensis*. Jour. Inf. Dis., **15**: 331-340. 1914.

A STUDY OF PATHOGEN-SELECTIVE CULTURES IN RELATION TO VACCINE THERAPY*

FRED BOERNER AND MYER SOLIS-COHEN

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Philadelphia*

The Solis-Cohen pathogen-selective method for preparing autogenous vaccines is based upon the assumption that organisms capable of growing in the fresh, whole, coagulable blood of the patient are those which are most pathogenic for that individual. This assumption followed the discovery that the whole blood of animals naturally resistant or immune to pneumococci¹ and to meningococci⁴ is bactericidal for such organisms, while the blood of animals naturally susceptible to them lacks this bactericidal property. One of the advantages of the method is that it furnishes a means for selecting the etiologically important organisms from a mixed culture. The pathogen-selective method is not applicable to spore-forming organisms, to those producing exotoxins, or to those difficult to cultivate, like the gonococcus, *Hemophilus influenzae*, et cetera.

The method consists of two simultaneous inoculations of the material to be cultured, one in a rich medium, such as Rosenow's brain broth, and the other in the patient's fresh, whole, coagulable blood *in vitro*.^{5, 6, 7, 8} After a primary incubation of twenty-four hours, both cultures are examined and the organisms present in each are studied for identification. The organisms which appear in the blood are those elected to predominate in the vaccine.

The present study consists of 404 pathogen-selective cultures from 150 patients. The Solis-Cohen technic, described in detail by Kolmer and Boerner,² was used. The majority of the cultures

* Read before the Joint Session of the American Association of Immunologists with the American Association of Pathologists and Bacteriologists, April 28, 1932.

were from the noses and throats of patients with subacute and chronic upper respiratory infections. Material from the rhinopharynx and from the tonsils, or tonsillar fossae, were often mixed and cultured together. The source and number of cultures are listed in table 1.

TABLE 1
SOURCES OF 404 PATHOGEN-SELECTIVE CULTURES FROM 150 PATIENTS

SOURCE	NUMBER
Nares.....	163
Nasal accessory sinuses.....	9
Tonsillar fossae and rhinopharynx.....	108
Tonsils and rhinopharynx.....	58
Tonsillar fossae.....	1
Tonsils.....	5
Rhinopharynx.....	5
Teeth.....	15
Sputum.....	19
Ear.....	3
Abscess.....	5
Urine.....	4
Feces.....	6
Anal fissure.....	1
Prostatic secretion.....	1
Carcinomatous ulcer.....	1

TABLE 2
GROUPING OF CASES ACCORDING TO RESULTS OF PATHOGEN-SELECTIVE CULTURES

GROUP	NUMBER
1. Same organisms in broth culture and in patient's blood.....	146 (36.1%)
2. Several organisms in broth culture but not all growing in patient's blood.....	108 (26.7%)
3. Organisms in broth culture but none growing in patient's blood.....	104 (25.7%)
4. Organisms growing in patient's blood but none in broth culture.....	46 (11.5%)

All of the cases studied have been classified, according to the results obtained, into four groups as shown in table 2. In approximately one-third of the cases the results were identical in both the blood and the broth, as shown in group 1. In the case

of group 2, which constituted about one-fourth of the cases, only certain of the organisms present in the broth grew in the blood. These were selected to predominate in the vaccines. The results in group 3 suggest the possibility that the organisms of etiological importance were not present in the material cultured or had been missed due to error in technic. The tests in these cases were repeated and if the same result was obtained, other foci were looked

TABLE 3
ORGANISMS PRESENT IN 404 PATHOGEN-SELECTIVE CULTURES FROM 150 PATIENTS

ORGANISM	NUMBER OF STRAINS ISOLATED	NUMBER AND PERCENTAGE WHICH GREW IN PATIENT'S BLOOD		NUMBER AND PERCENTAGE WHICH DID NOT GROW IN PATIENT'S BLOOD
		%	%	
Streptococci (hemolytic).....	32	25 (78.1%)	7 (21.8%)	
Streptococci (viridans group).....	63	41 (65.0%)	22 (34.9%)	
Streptococci (non hemolytic).....	160	98 (61.2%)	62 (38.7%)	
<i>Staphylococcus aureus</i>	113	82 (72.5%)	31 (27.4%)	
<i>Staphylococcus albus</i>	182	64 (35.1%)	118 (64.8%)	
<i>Staphylococcus pharyngis</i>	3	1 (33.3%)	2 (66.6%)	
Micrococci (unidentified).....	8	2 (25.0%)	6 (75.0%)	
<i>Neisseria catarrhalis</i>	15	0	15 (100%)	
<i>Neisseria sicca</i>	21	0	21 (100%)	
<i>Neisseria perflava</i>	1	0	1 (100%)	
Gram negative cocci (unidentified)...	19	4 (21.0%)	15 (78.9%)	
<i>Diplococcus pneumoniae</i>	22	9 (40.9%)	13 (59.0%)	
<i>Corynebacterium pseudodiphthericum</i>	21	9 (42.8%)	12 (57.1%)	
<i>Klebsiella pneumoniae</i>	8	4 (50%)	4 (50.0%)	
<i>Escherichia coli</i>	9	4 (44.4%)	5 (55.5%)	
<i>Proteus vulgaris</i>	3	3 (100%)	0	
Gram negative bacilli (unidentified)...	21	2 (9.5%)	19 (90.4%)	
<i>Hemophilus influenzae</i>	3	0	3 (100%)	
<i>B. subtilis</i>	8	2 (25.0%)	6 (75.0%)	
Yeast.....	3	1 (33.3%)	2 (66.6%)	

for and cultured, especially if evidence of systemic infection existed. Group 4 shows that in 11.5 per cent of the cases organisms appeared in the blood, which would have been missed had only broth cultures been made.

The various organisms isolated and the frequency with which they appeared in the blood and in the broth cultures are listed in table 3.

A study of this table shows that organisms usually classed as pathogenic for man were the ones which grew most frequently in the patient's whole coagulable blood, namely streptococci and staphylococci. It is to be noted that the hemolytic strains of streptococci showed the highest percentage growing in the patient's blood and that the *staphylococcus aureus* grew more frequently in the blood than did other members of this group. The opposite was true of the Gram-negative cocci, which are slightly, if at all, pathogenic. Of these but four of the fifty-six cultures grew in the patient's blood. The pneumococcus grew in the blood nine times and failed to grow thirteen times. These strains were not typed but, being from non-contact cases, they probably belonged to group IV.

It is difficult to explain why such a high percentage of the diphtheroids grew in the patient's blood. Fifty per cent of Friedländer's pneumobacillus and four of the nine strains of *Esch. coli* (*B. coli*) grew in the blood. The occurrence of *B. subtilis* in the blood was no doubt due to the presence of resistant spores in the material cultured, which remained viable in the blood and grew upon subculturing. The unidentified Gram-negative bacilli were only studied sufficiently to prove that they were not of the pathogenic types. These were considered saprophytic and, as shown in the table, only two of the twenty-one strains grew in the patient's blood. The other organisms appeared too infrequent to permit of deductions or conclusions.

DISCUSSION

The data in this series of pathogen-selective cultures are very similar to those reported by Lowe.³ He reported upon 600 pathogen-selective cultures and classified his patients into five groups. His group of cultures giving similar results, which corresponds to our group 1, contained only 6 per cent. This difference may be due to the source of the cultures and to the types of the infections studied. Lowe made the statement that although apparently non-pathogenic organisms were present in a large number of cases; only in one of the thirty-four examinations did such an organism as *Neisseria catarrhalis* (*Micrococcus catarrhalis*) appear

in the blood-controlled culture. Our results as a whole are very similar to those of Lowe.

The question arises as to whether the pathogenic organisms which failed to grow in the patient's blood should be considered as entirely harmless or as capable of causing local irritation and inflammation, but not systemic infection. In this connection we have also to consider the possibility of bacteria producing systemic diseases by the absorption of their toxins from areas of local infection. Lowe was of the opinion that these organisms are capable of causing local but not systemic infection. These questions are debatable and with the present knowledge can not be definitely answered. In view of this fact we have not entirely ignored the pathogenic bacteria which failed to grow in the blood, but have included them in the vaccines to the extent of 10 per cent. The following is the manner in which the organisms were selected for vaccines:

In Group 1 the organisms growing in the blood rather than those growing in the broth were selected for the vaccine, even though they appeared identical.

In Group 2 90 per cent of the organisms which grew in the blood and 10 per cent of the pathogens which grew in the broth, but failed to grow in the blood, were included in the vaccine. Organisms considered as non-pathogenic, which grew in the broth only, as well as spore-bearers were not included.

In some cases of Group 3 vaccines were prepared from the broth cultures for the purpose of combating only the local infection. Where evidence of systemic infection was present other foci of infection were looked for and studied.

Group 4 was considered the same as group 1.

The clinical results of the vaccines prepared from the cultures reported in this paper will be the subject of a future publication by one of us (M. S-C).

The pathogen-selective method of culturing has also been found useful by one of us (F. B.) as an aid in isolating such organisms as the streptococci from mixed infections and contaminated material. The patient's fresh, whole, coagulable blood in such cases very often retards or inhibits the growth of the unimportant

organisms which overgrow in the ordinary culture. As an example, a nasal culture received for the preparation of a vaccine, was overgrown with *Proteus vulgaris* but in which streptococci were recognized in smears. The culturing was repeated upon two occasions with the same results. It was then decided to inoculate the nasal secretions into the patient's blood. This was done, with the result that streptococci and staphylococci were isolated without interference with *P. vulgaris* as the blood proved bactericidal for this organism and thus eliminated it from the culture.

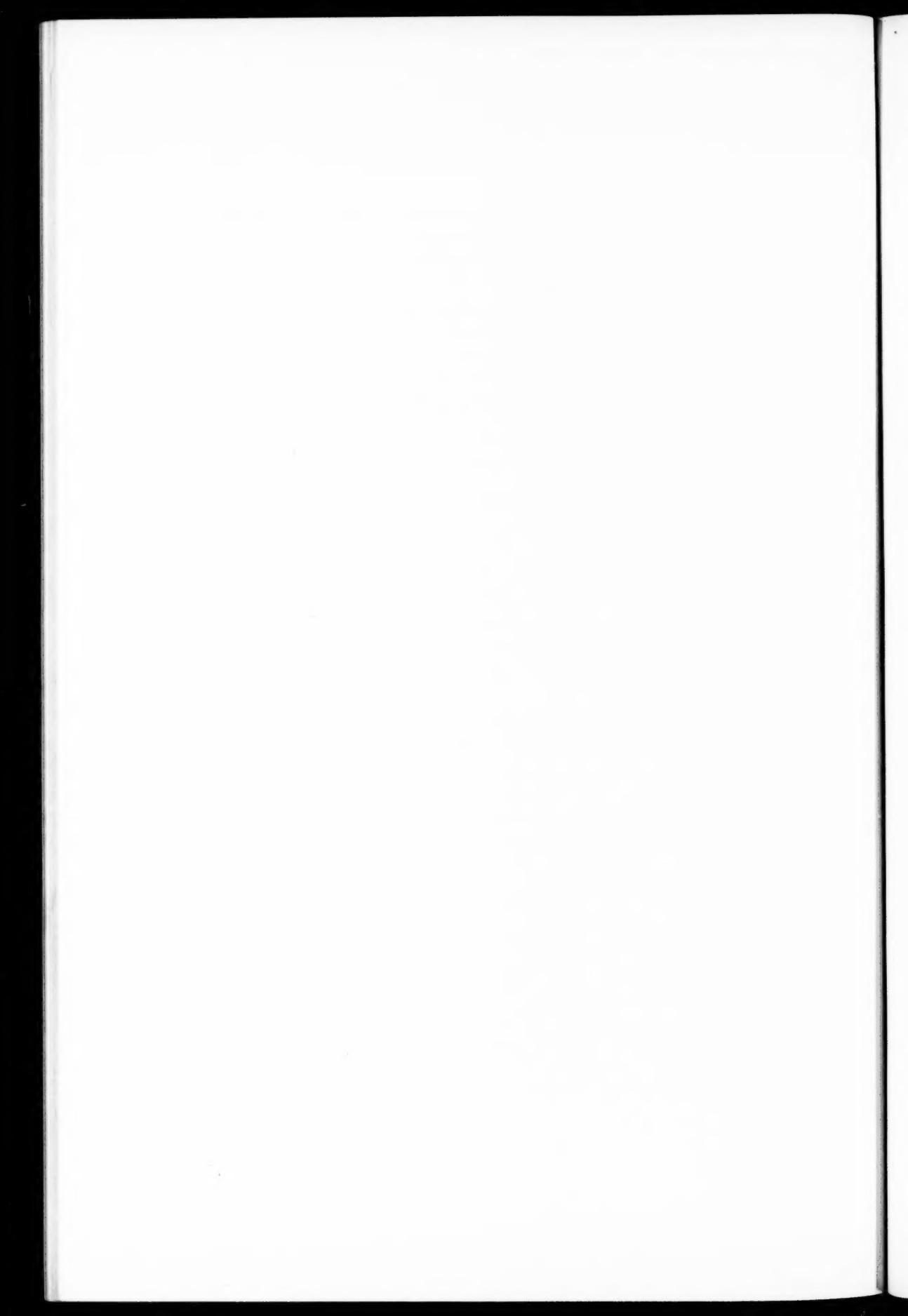
CONCLUSIONS

- (1) A series of 404 pathogen-selective cultures from 150 patients were studied.
- (2) In many cases the isolation of the more important pathogenic organisms was aided by the bactericidal action of the patient's fresh, whole, coagulable blood upon the less important and presumably non-pathogenic organisms.
- (3) Information was obtained regarding the presence or absence of bactericidal substances in the patient's blood for the various organisms isolated.
- (4) In about 11 per cent of the cases organisms were isolated which would have been missed by the ordinary methods of culturing.
- (5) A basis is provided for selecting the organisms to predominate in an autogenous vaccine, on the assumption that the organisms which grow in the patient's fresh, whole, coagulable blood are of most importance to the individual.

REFERENCES

- (1) HEIST, G. D., SOLIS-COHEN, SOLOMON AND SOLIS-COHEN, MYER: The bactericidal action of whole blood, with a new technique for its determination. *Jour. Immunol.*, **3**: 261-276. 1918.
- (2) KOLMER, J. A., AND BOERNER, FRED: Approved laboratory technic. New York, D. Appleton & Co., 1931. 663 pp.
- (3) LOWE, E. C.: Pathogen-selective cultures as an aid to the diagnosis of infective foci. *Brit. Med. Jour.*, **2**: 98-100. 1928.
- (4) MATSUNAMI, TOITSU, AND KOLMER, J. A.: The relation of the meningo-

- coccidal activity of the blood to resistance to virulent meningococci. *Jour. Immunol.*, **3**: 201-212. 1918.
- (5) SOLIS-COHEN, MYER: Accentuating pathogenic organisms in culture by utilizing inhibitory influence of whole blood. *Brit. Exp. Path.*, **8**: 149-154. 1927.
- (6) SOLIS-COHEN, MYER: The pathogen-selective vaccine: its preparation and administration. *Med. Times*, New York., **58**: 206, July 1930.
- (7) SOLIS-COHEN, MYER, AND HEIST, G. D.: A method of distinguishing from among various microorganisms present in a patient, those that are and those that are not acted upon by that patient's whole coagulable blood. *Penn. Med. Jour.*, **25**: 27-34. 1922.
- (8) SOLIS-COHEN, MYER, AND RUBENSTONE, A. I.: The technic of the pathogen-selective method of culture. *Jour. Lab. and Clin. Med.*, **11**: 881-886. 1926.



A STUDY OF O AND H AGGLUTININS IN TYPHOID AND ENDEMIC TYPHUS FEVER*

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The applications of the work of Felix³ in qualitative receptor analysis to problems of serum diagnosis in the enteric fevers, particularly typhoid, has so far engaged the attention of English and continental investigators that today they hold rather definite conceptions of the situation in regard to the serum diagnosis of typhoid, particularly in the inoculated individual. The finding of H, or flagellar antibodies, in the serum of previously inoculated individuals has been so constant since the first reports by Felix; Topley, Platts and Imrie; Rosher and Fielden, and MacVie and Smith, "That we cannot assess the significance of a positive agglutination test with any certainty if we know that the patient has received a previous prophylactic inoculation, or if we are uncertain on that point."⁵ Topley and Wilson⁶ offer two ways of escape from this apparent dilemma: The application of the principles of qualitative receptor analysis (Felix), and serial quantitative estimations, the so-called comparative Widal test of Dreyer. The encouraging results with the qualitative methods published by the English workers mentioned above, and the more recent reports by American investigators, Eldering and Larkum² led to this study.

In regard to the H agglutination, this study brings out no essential differences in the now well established mechanism governing this phenomenon (Craigie¹). As has been observed many times before, it was noted that the serum of typhoid vaccinated individuals, in typhoid cases, in endemic typhus cases and in cases of undulant

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fever agglutinated (sometimes at high dilution) the 0.1 per cent formolized broth cultures of typhoid bacilli accepted as H antigen. Our main interest, therefore, became centered upon the problem of O agglutination as applied by Felix to the diagnosis of typhoid in the some time inoculated cases. Felix^{3,4} strongly contended that the individuals inoculated with typhoid-paratyphoid vaccine do not develop O agglutinins for typhoid organisms which have been selected in their O phase of growth. Instead of the phase-selected organisms the use of mechanically deflagellated bacteria, or alcoholized bacteria, has been so successful in the hands of so many English investigators that the Topley and Wilson text accepts the alcoholized typhoid bacilli as O antigen as fully as the formolized broth cultures for H antigen. It appears that such an O antigen may be prepared by treating thick emulsions of typhoid bacilli with 50 per cent alcohol and subsequently diluting this antigen to the desired turbidity. It is necessary that the culture so treated be definitely S type organisms since S→R dissociation brings about a change in the specific somatic (O) substance to a non-specific agglutinogen (\emptyset) found to be shared by unrelated species of bacteria. Alcohol may definitely affect the reaction, but it has been found that a final concentration of as much as a 5 per cent alcohol in the tests has no effect upon the reaction.

TECHNIC

The following O antigens were used: a 50 per cent alcoholized emulsion diluted to standard turbidity, final alcohol concentration 2.5 per cent; a 10 per cent alcoholized eighteen hour broth culture, final alcohol concentration 2.5 per cent; a culture of growth phase O antigen (Felix's Ty O-901 obtained through the courtesy of Dr. N. W. Larkum, who obtained it from Drs. W. W. C. Topley and A. D. Gardner of the British Medical Research Council) and the same culture, Ty O-901, treated with 0.2 per cent formol.

The idea in using the 10 per cent alcoholized antigen was to find out if the broth cultures so treated might be usable and so make possible the elimination of the steps necessary to preparing thick emulsions.

With regard to the formolizing of the O growth phase culture, it is to be noted that despite the fact that Topley and Wilson commented rather frequently in their text on the depressing activity of formalin on O agglutination, and in spite of the fact that Felix⁴ had shown definitely that formalin does depress the activity of the somatic agglutinin, the results of Eldering and Larkum² tended to

show that the presence of 0.1 per cent formol in the Ty O-901 culture does not sufficiently depress the agglutination of this selected O antigen to the extent that there is definite interference with agglutination.

The sera employed in this study came from four groups of individuals. The first was a group of six cases of proven typhoid fever, three of whom had had, three to five years previously, typhoid-paratyphoid vaccine, and three who had not had typhoid-paratyphoid vaccine. It was possible to follow their serum reactions throughout the course of their illnesses. A second group was composed of fifteen cases of typhoid in which at least two samples of sera were obtainable during the course of their illnesses. The third and fourth groups were, respectively, thirty typhoid-paratyphoid vaccinated Reserve Officers Training Corps students, and thirty individuals whose sera were obtained for another problem, a study in chronic arthritis.

In carrying out the tests equal parts of antigen and serum (1 cc.) were incubated at 37 degrees centigrade for eighteen hours, following which the tests were placed in the ice-box for two hours before reading.

A SERIAL STUDY OF SIX TYPHOID CASES

In the study of the six typhoid cases, serum taken within the first week failed to agglutinate any of the O antigens. In the second week it was found that both alcoholized antigens were agglutinated in all six cases at the following low dilutions: 1:20, 1:20, 1:40, 1:20, 1:60, and 1:80. The growth-phase antigen, Ty O-901, and the formolized antigen were not agglutinated at this time, nor during the next, the third week. During the third week the agglutinating titers for the alcoholized antigens rose perceptibly, becoming 1:80, 1:160, 1:160, 1:160, 1:240, 1:340, in the order first named.

A 0.1 per cent formolized broth culture of *Eberthella typhi* used as a sort of control in these tests was agglutinated for the first time (the third week) in serum from cases 4, 5, and 6, in dilutions of 1:40, 1:80, and 1:60 respectively. In the fourth week the agglutinating titers for the alcoholized antigens reached the following high levels: 1:320, 1:640, 1:480, 1:640, and 1:640. The growth phase O antigen was agglutinated at some dilution by all of the sera during the fourth week. The titers were, however, very low compared to the high titers with the alcoholized antigens. They were 1:40, 1:40, 1:20, 1:30, 1:60, and 1:40 respectively. The formolized growth-phase culture was agglutinated by one

serum only, number 6, at a dilution of 1:40. The formolized culture of *E. typhi* was not agglutinated by the sera of cases 1, 2, and 4, while the sera of cases 3, 5, and 6, increased in agglutinating

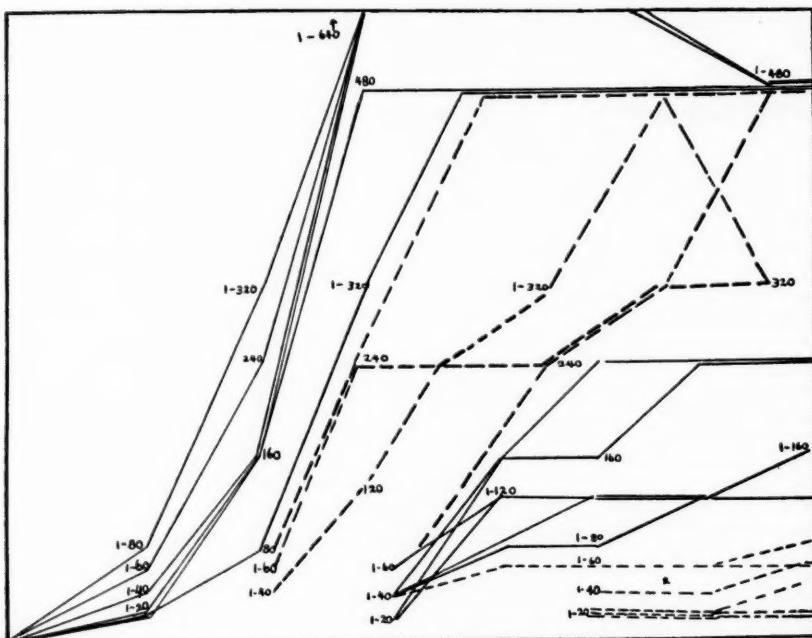


FIG. 1. AGGLUTINATING TITERS IN SIX CASES OF TYPHOID FEVER STUDIED DURING A PERIOD OF EIGHT WEEKS

- (1) The unbroken lines at the left show the agglutinating titers of the patients' sera with alcoholized (artificial O) agglutinogen.
- (2) The broken lines in the center show the agglutinating titers with 0.1 per cent formolized *E. typhi*.
- (3) The unbroken lines in the lower right represent the agglutinating titers with Felix's Ty O-901 (growth-phase selected O agglutinogen).
- (4) The broken lines in the lower right represent the agglutinating titers with 0.1 per cent formolized Ty O-901.
- (5) The numerals (e.g. 1-320) represent the dilutions of the patients' sera found to agglutinate the various antigens at weekly intervals beginning with the second week of the illness.

titer for this antigen to 1:120, 1:240, 1:340 respectively. During the fifth week the alcoholized antigens were still agglutinated at relatively the same levels. The growth phase O antigen was

agglutinated at increasingly higher levels: 1:80, 1:80, 1:120, 1:160, 1:120, 1:160, and the formalized growth phase O antigen was agglutinated by two sera, numbers 4 and 6, at dilutions of 1:60 and 1:60 respectively. The formalized culture of *E. typhi* was agglutinated by four sera, 3, 4, 5, and 6, at dilutions of 1:240, 1:80, 1:240, and 1:480, respectively. Serum from cases 1 and 2 still failed to agglutinate the formalized culture of *E. typhi*.

In the sixth week there were no essential changes in the agglutinating titers except in the case of the formalized growth phase O antigen wherein some agglutination was noted in each case, but all of these were at a relatively low titer, namely 1:20, 1:20, 1:20, 1:60, 1:40, and 1:60. Cases 1 and 2 were still negative to the formalized broth culture of *E. typhi*. Cases 1, 2, 3, and 4 were making fair progress toward recovery and cases 5 and 6 were nearing convalescence.

In the seventh week after the onset of the disease, serum was again tested in these six cases. No essential changes from the sixth week were found.

In the eighth week serum obtained from the six patients, four of whom were definitely convalescent, the other two nearly so, showed but little change from the sixth and seventh week. Agglutinating titers for the formalized growth-phase cultures were slightly higher on the whole, and the serum of case number 2, which had been persistently Widal negative throughout the course of the illness, at this time partially agglutinated the formalized broth culture of *E. typhi* at a dilution of 1:40.

In this series cases 1, 2, and 3 were individuals who had never been vaccinated against typhoid and paratyphoid and who also gave no history of similar previous illnesses. Cases 4, 5, and 6 had had either full or partial vaccination at intervals varying between three to seven years prior to the onset of their illness. A brief history of their vaccination follows. Case 4 had had two doses of typhoid-paratyphoid vaccine three years prior to onset of present illness. Case 5 had had a full course of vaccine seven years previously. Case 6 had had one dose of vaccine a year and a half before the onset of the present illness.

All of these patients recovered. There were no serious compli-

cations in any of the cases studied. Convalescence in case 2 was prolonged some three weeks longer than in the others. Clinically, then, there were no very great differences in these cases. Serologically, the previously vaccinated group showed the presence of agglutinins sooner and at higher titer than the non-vaccinated group. In this connection the point to be emphasized particularly is the fact that the agglutination of the growth phase O antigen appeared later and at lower titers than the alcoholized or formolized typhoid antigens. The formolized growth-phase antigen was agglutinated only after a longer period of time and at relatively lower titers than even the growth phase O antigen. Typhoid-paratyphoid vaccination, apparently, had some effect upon the reactions. However, O agglutinins were found at practically the same time in the serum of both the previously vaccinated and the non-vaccinated.

The complete failure of one serum, and the partial failure of another, to agglutinate the H antigen is nothing new in the experience of individuals who have done many Widal tests in cases clinically typhoid. Felix⁴ reported 27 per cent of 531 proved cases of typhoid reacting negatively to Widal tests in which formolized typhoid cultures were used as antigen. In all of these cases positive agglutination of O antigen was observed. Felix also quotes Pijper as recording 28 per cent of 120 proved cases of typhoid in South Africa, which were persistently "Widal negative" to 0.1 per cent formolized broth cultures.

It appears, then, that somatic (O) antibodies are produced at some time during typhoid fever, however, in low titer, and that any attempt at serum diagnosis of typhoid which fails to include an antigen for their detection may fail because of the absence of H agglutinin in a rather high percentage of cases.

AGGLUTININS IN FIFTEEN TYPHOID CASES

In the second group of cases studied, fifteen in all, serum was obtainable only twice during the illness, the first samples being obtained within the second ten-day period of the disease, and the second samples being obtained within the third and fourth ten-day periods of the illness. Ordinarily we expect the Widal to

become positive at some time within the second ten-day period. Certainly in proved cases of typhoid we expect a high percentage of positive reactions by the time the third and fourth ten day periods have been reached.

Without detailing here the data in the fifteen cases in this group, but from taking the findings by and large, it appears that alcoholized antigens are agglutinated sooner and at remarkably higher titers than the growth-phase antigens whether the growth phase antigens have been formolized or not. The growth phase antigen, Ty O-901 was agglutinated by only five of the fifteen sera taken within the second ten-day period, and then at dilutions of 1:20, 1:40, 1:20, 1:80, and 1:40 respectively. Formolizing this culture depressed the reaction to lower levels of agglutinating titer, (1:20, 1:40, 1:20) and entirely eliminated agglutination in two of the five cases. The H antigen (0.1 per cent formolized broth culture of *E. typhi*) was not agglutinated by six of the fifteen sera taken within the second ten-day period.

The second tests in this particular series help to substantiate the impressions gained from the first. In this instance both of the growth-phase antigens were agglutinated at some titer. However, this was very low when compared to agglutination titers usually found at this time. In this, the final test in this group, all but three cases in fifteen agglutinated the H antigen at titers above those usually considered diagnostic for typhoid fever.

History of some sort of previous vaccination was obtainable in five of the fifteen cases, no one of which was more recent than five years.

AGGLUTININS IN VACCINATED INDIVIDUALS

In order to determine whether or not typhoid-paratyphoid vaccination produced O agglutinins in the inoculated individual, serum was taken from thirty medical students who were to be vaccinated against typhoid-paratyphoid prior to going to summer training camp. Thirteen of these men claimed they had never received typhoid-paratyphoid vaccine, nor had had typhoid fever. Testing their sera with the five antigens used in the previous experiments it was found that all but five of them agglutinated the

alcoholized antigens at some dilution ranging between 1:20 and 1:240, the average falling between 1:80 and 1:120. On the other hand only five agglutinated the growth phase O antigen and at dilutions of 1:20, 1:20, 1:40, 1:20, 1:40, respectively. None of them agglutinated the formolized growth phase O antigen (Ty O-901, plus 0.1 per cent formol). Of the seventeen who reported previous vaccination in some amount, all of them agglutinated the alcoholized antigens at some titer between 1:40 and 1:640. All but six failed to agglutinate the growth phase O antigen, and ten failed to agglutinate the formolized growth phase O antigen.

These results are not clearly differential. They might have some bearing on the situation if the investigation could have been stopped at this point. But when it was found at two, four, and six weeks following the routine vaccination administered by the medical officer in charge of the Reserve Officers Training Corps work at Baylor University, that the serum of all of the previously non-vaccinated men now agglutinated all of the O antigens at some titer between 1:120, and 1:640, it became apparent that O agglutinins are produced by the typhoid-paratyphoid vaccine prepared by the U. S. Army Medical School. There is little reason to hope, therefore, that typhoid can be diagnosed simply by determining the presence of O agglutinins in the sera of previously vaccinated individuals.

TYPHOID AGGLUTININS IN CHRONIC ARTHRITIS

The opinion stated above seems to be substantiated in the findings from a similar study carried out with serum from cases of chronic streptococcic (?) arthritis taken during periods of acute exacerbation. These sera were taken for another study but were available for this particular investigation of the possibility of the presence of O agglutinins in the serum of individuals acutely ill with some disease other than typhoid.

Thirty samples of such serum were studied. Thirteen of the thirty had had a full or partial typhoid-paratyphoid prophylaxis at times ranging between two and fourteen years previously.

Seventeen denied ever having had typhoid-paratyphoid vaccination.

Only two of the thirty sera from individuals previously vaccinated and not previously vaccinated, failed to agglutinate at some dilution between 1:80 and 1:1280, either or both of the alcoholized antigens. The average agglutinating titer in this series was between 1:160 and 1:240. Furthermore, none of the serum from the non-vaccinated cases agglutinated the "H" antigen, and only five of the thirteen vaccinated cases furnished sera which agglutinated the H antigen in dilutions more than 1:40.

The results in this series with the growth-phase antigens do not allow any sweeping conclusions. The serum from a small group (three) of the vaccinated cases agglutinated the formolized growth phase culture at dilutions of 1:40, 1:20, 1:20, respectively, while the serum of a somewhat larger group (seven) of the vaccinated cases agglutinated these antigens at much the same titer. None of the samples of serum from the non-vaccinated cases agglutinated the growth phase antigens, formolized or non-formolized.

DISCUSSION

From these somewhat contradictory findings it does seem to be apparent that O agglutination can and does take place under conditions other than typhoid infection. It seems, therefore, that the diagnosis of typhoid fever cannot be made simply upon the demonstration of the presence of O agglutinins in the patient's serum.

Topley and Wilson held that a case may be made for this method if a sufficiently higher titer of agglutination be set as a standard. Such a test would demand: first, the use of a rigidly standardized antigen both for qualitative and quantitative principles, and, second, a titer high enough to rule out completely the "positive" findings of non-specific origin.

The level which they proposed as diagnostic is 1:100, but from my study it seems that this would allow the test to be of value only after several weeks of the illness. Then, too, it must be recalled that many of the group of Reserve Officers Training Corps students in this study developed O agglutinins for the

growth-phase antigen to even higher titers than 1:100 following their vaccination. For this reason the possibility of the serum of a previously vaccinated individual developing a high titer of agglutinins to an infection other than typhoid must not be overlooked.

It further appears that artificially prepared "O" antigens are of no value unless especially high titers are to be taken as diagnostic and even then it would seem necessary in this instance that the comparative tests (Dreyer) be used to show that such a reaction might not be due entirely to anamnestic reactions observed at the onset of any infection.

It is neither the purpose nor the result of this study to make a case against O antigens and O antibodies. Certainly the constant finding of some amount of O agglutination during the course of an illness otherwise impossible to diagnose as typhoid is of great value, and may go a long way toward clearing up the now troublesome ambiguities of the Widal test.

AGGLUTININS IN ENDEMIC TYPHUS

With regard to the somatic and flagellar antibodies in endemic typhus fever, the material available for a study comprised only seven cases, in each of which one or two Weil-Felix tests were done using 0.1 per cent formolized suspensions of *B. proteus X19* as antigen.

All of these cases were, clinically, endemic typhus in which the diagnosis was firmly supported by positive MacNeil (scrotal swelling) reaction in male guinea-pigs inoculated intraperitoneally with 10 cc. of fresh blood from the patient. In all of these cases positive agglutination of the formolized (H) antigen was observed as titers above 1:240, and in one instance as high as 1:2560. Identical agglutinations were obtained with 2.5 per cent alcoholized broth cultures of *B. proteus X19*. Felix⁴ stated that epidemic typhus causes the formation of O agglutinins only which are not detectable by formolized antigen. In view of our findings in endemic typhus in north Texas it might be a point of differentiation between the two diseases, Old World and New World typhus,

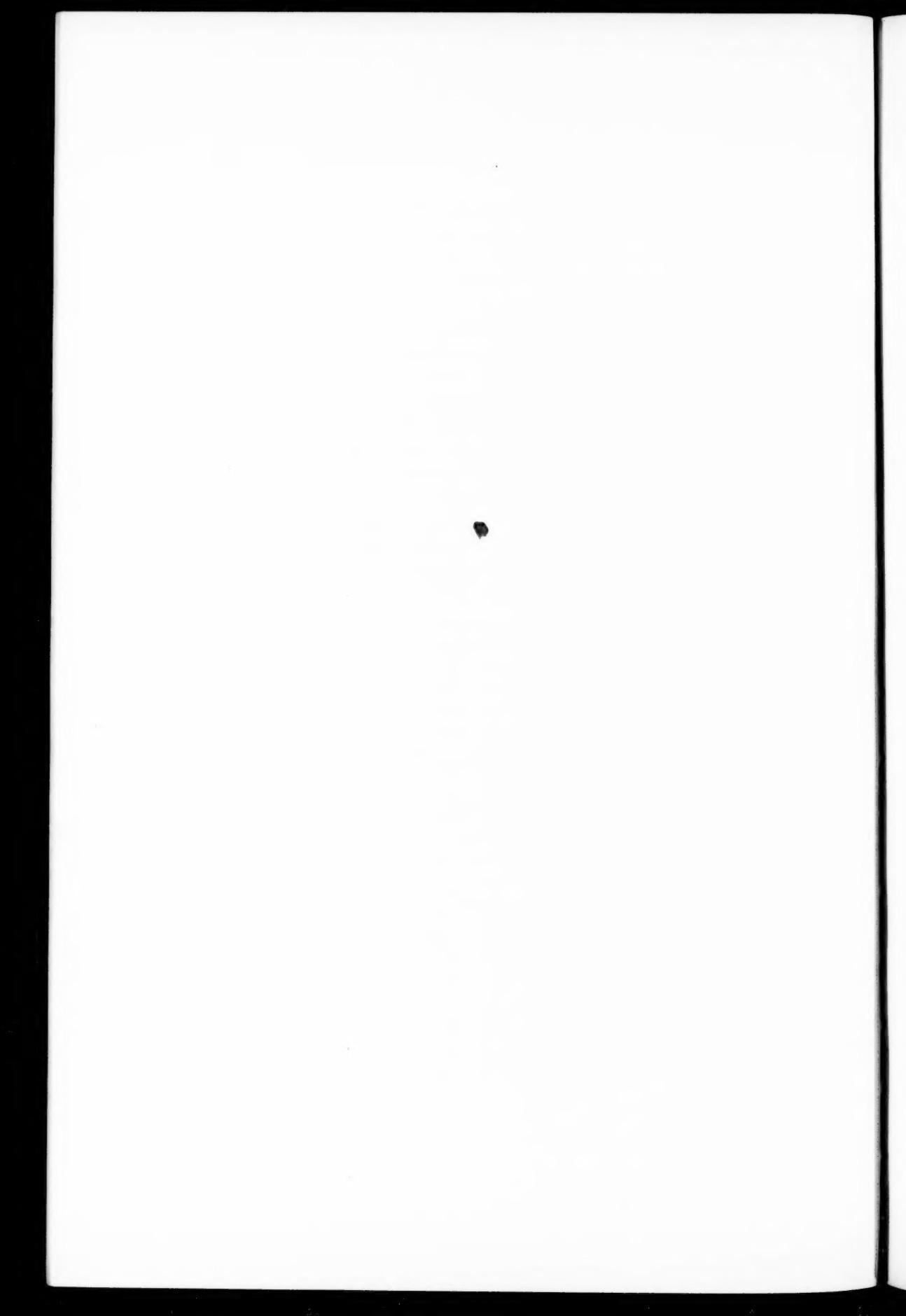
to observe that endemic or New World typhus seems to cause the development of both O and H agglutinins for *B. proteus X19*.

SUMMARY

1. Alcoholized *E. typhi* were used as O agglutinogen in agglutination tests carried out serially in six cases of typhoid fever. Positive agglutination was observed earlier than is usually observed in the Widal test.
2. Growth-phase selected O agglutinogen was positively agglutinated later than is usually found in the Widal reaction.
3. Formolized growth-phase O agglutinogen was agglutinated even later than untreated growth-phase selected agglutinogen and at much lower titers.
4. Both O and H agglutinins were found in the sera of typhoid-paratyphoid vaccinated individuals. The same individuals did not have O agglutinins prior to vaccination.
5. Both O and H agglutinins were found in the sera of individuals suffering with acute exacerbations in chronic arthritis.
6. Both O and H agglutinins for *B. proteus X19* were found in the serum of seven cases of endemic typhus (north Texas).

REFERENCES

- (1) CRAIGIE, JAMES: Studies on the serological reactions of the flagella of *B. typhosus*. *Jour. of Immunology*, **21**: 417-512. 1931.
- (2) ELDERING, GRACE, AND LARKUM, N. W.: Antigens in the Widal. *Am. Jour. of Public Health*, **21**: 1370-1374. 1931.
- (3) FELIX, A.: The qualitative receptor analysis in its application to typhoid fever. *Jour. of Immunology*, **9**: 115-192. 1924.
- (4) FELIX, A., AND OLITZKI, L.: The use of preserved bacterial suspensions for the agglutination test with special reference to enteric fevers and typhus fever. *Jour. of Hygiene*, **28**: 55-66. 1928.
- (5) TOPLEY, W. W. C., AND WILSON, G. S.: *The principles of bacteriology and immunity*. New York, William Wood and Company, 1929.



EXPLORING DEATH*

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It is indeed an honor and a privilege to dedicate a department founded for the purpose of obtaining knowledge and disseminating wisdom regarding the afflictions decimating man and thus to lead to a prolongation of happiness and life through such information. I might remind you briefly that man's view of death is flavored by his knowledge of the subject. Our colleague and recent contemporary, Aldred Scott Warthin, of the University of Michigan, calls life a tragicomedy in three acts: I. Evolution; II. Maturity; III. Involution, synonymous with infancy and youth, maturity and senescence each with individual characteristics. He points to the biologic span of life and its termination in normal or biologic death but this is not the only form of death that may come to the multicellular animal organism, nor is it the usual one. Unfavorable factors in the environment may check the career of the individual at any time in its course—pathologic extrinsic death, the most common fate of animal life, or inherent abnormalities may be present in the germ-plasm of any given line foreordaining its early or premature termination—pathologic intrinsic death (inherited). Very few, if any, human beings achieve a biologic span of life and a normal intrinsic death; the great majority succumb to a pathologic extrinsic death, a smaller number to a pathologic intrinsic death.

Death is of universal interest to the ecclesiast, to the poet, to the musician, to the artist, to the jurist, to the mother and father, and most to the physician. The latter assists at birth and is

* Address read at the dedication of the Department of Pathology and the opening of the new autopsy room of the School of Medicine of the Louisiana State University and the Charity Hospital Medical Center. The dedication was made on May 9, 1932 by the American Society of Clinical Pathologists, Dr. H. J. Corper, President, Dr. A. S. Giordano, Secretary-Treasurer. The laboratories of Pathology are under the direction of Dr. Rigney D'Aunoy.

continually engaged in preventing death, the two most important periods of life. Physicians and scientists through centuries of investigation laid the foundation for modern man's rational acceptance of death, bringing the conception from a gruesome tragedy to the present day idea. It is an ever changing panorama of life which has found expression variously in man's emotions. The Toten-Tanz or Danse Macabre (Dance of Death) Motive which for more than half a thousand years had an extraordinary vogue in the literature and art of Middle Europe is illustrative. Its origin is unknown; we do not know the name of the artist who first painted a Dance of Death, or that of the poet who produced the first literary form of the Motive. Probably originally it was a church play, performed within the church or churchyard, for the religious didactic instruction of the people. During the early Christian centuries the *carpe diem* philosophy of the ancients gradually became replaced by that of the Christian *memento mori*. Faith became superstition in the Dark Ages among the uneducated and ignorant people; the remnants of learning and spiritual life were found only in the church; the laity could scarcely read or write. In order to enforce its teachings of a moral life, the church found that its most efficient method was in the emphasis laid upon death, the last judgment, and the alternative future of heaven or hell. The most satisfactory explanation of the origin of the Dance of Death motive is to be found in the psychology of the times. The Middle Ages felt the primitive horror of death and expressed it in the form of the putrescent cadaver. Death was thought of only in its horrible and gruesome aspects, of the consolations of death the Middle Ages had no conception; the fear of the agony of death transcended all other emotions. To the mind of the period, the visions of the apocalypse made special appeal. What else could be expected from minds exposed to daily contact with the danger of death; in the cities during these centuries pestilence almost day by day claimed its victims by the thousands. Out of all these sources, liturgy, sermons, mystery plays, legends and poems, together with the morbid psychology and superstitions of the people there evolved a great folk cultural idea which took form in the Dance of Death as expressed in the

great wall paintings and woodcuts presenting a satire of social equality and its significance in the cultural evolution of modern society and which cannot be disregarded. The main themes of the Dance of Death are as potent today as ever they were, though altered in their significance; and they will retain their value for the human mind as long as the race persists. The physician played a potent part in all this and is revealed as he appeared to the layman, and in the latter's opinion of him. The changing social standing, professional manners and mannerisms, the progress in the knowledge and the practice of medicine and a true picture of the type of man who became a physician is revealed. Finally, in modern art we have catalogued the different forms in which death lurks for his victims. The progress made in the material world, railway trains, motor cars, airplanes, reflect new forms of death. The modern has lost his fear of death and meets it with resignation or bravery, or with cynical indifference and to this the physician and scientist has contributed. Better understanding, painless operations, bacteriology and disinfection, decrease in contagious and infectious diseases, finally a gain in knowledge of the true state of fatal human ills by more post-mortems and removal of superstitions and prejudices has brought us to the present views.

In music there have appeared many short themes of death but none can approach in picturesqueness the symphonic poem "Danse Macabre" from the pen of the talented French composer, Camille Saint-Saëns (1835-1921) inspired by the verses by Henri Cazalis (1840-1909), a poet with a penchant for gloomy and grotesque subjects. The theme is that of a dance of death from the stroke of twelve to the crow of the cock announcing the approach of day.

Inspired by the famous painting (1880) of the Swiss-German artist, Arnold Böcklin, Sergei Rachmaninoff, the Russian-American composer, wrote (1906) the remarkably descriptive and unusually beautiful tone poem "The Isle of Death" with the "Dies Irae" theme of antiquity interwoven.

Dies irae dies illa,
Solvet saeculum in favilla,
Teste David cum Sibylla.

The day of wrath, that dreadful day
Shall the whole world in ashes lay,
As David and the Sibyl say.

The latter theme (*Dies Irae*) was also introduced by Ernest Schelling, the outstanding modern American composer in "The Victory Ball" (1922) based on Alfred Noyes' poem of this name, after returning from Europe still very much under the impression of the cataclysm and finding so few remembered what the war really had meant, with its sacrifice of life and youth. A brilliant polonaise is first heard with the rhythm of the fox trot and tango mingling through it. Then there is a dramatic interruption as the vision of the dead appears before us, then two trumpet calls and the *Dies Irae* and revel again commences. Through its sensuous strains the procession of the dead continues. The bag pipes of a Scotch regiment pass and a mighty fortissimo develops. Then there is a long roll on the drums and a trumpeter sounds "Taps."

Death has held a large part of the arena even in modern art. In the Widener Memorial Library at Harvard University at Cambridge, Massachusetts, is seen a beautiful example of the conflict between death and victory, a mural painting by John Singer Sargent, the genealogical product of New England. While victory claims the hero for apotheosis death holds him for its own. How well the pathologist could look upon this theme as making life safe through exploring death, a suggestion for an artist and an analyst!

Death can be conceived as a natural and physiologic process, necessary in the scheme of existence. It can be accepted as such, and happy is the man who can do so. The Dance of Death idea is as immortal as the life of the race; but in each new period of human thought it will express itself in new form corresponding to the predominant philosophy of that time. Behind that philosophy will be seated the material knowledge and wisdom of the pathologist, the scientist, and the physician. His work will be to explore death in its dynamic aspects.

I have purposely refreshed your memory on the emotional side of death as an introduction but no narrative of death would be complete at present without introducing you again to a few of the pioneer explorers of death and to the part such explorations have played in our medical knowledge as well as to the absolute neces-

sity of every medical student and physician being groomed in this subject.

Just when postmortem examination or the exploration of dead bodies began for the sake of learning about disease, it is difficult to say but we do know that there were always small groups of students who for the sake of gaining knowledge were willing to risk punishment at the hands of then existing laws. Ancient physicians recognized many diseases but their knowledge of the human body was so scant that they were incapable of comprehending the significance of the mechanisms of many diseases beyond the fact that they produced death. Their theories were usually all wrong. Galen (130-200 A.D.), as you remember, was the first to dissect the muscles and describe them (in apes). He also wrote on the physiology and pathology of the body but in the light of modern experimental medicine his hypotheses and system of medicine have been completely overthrown.

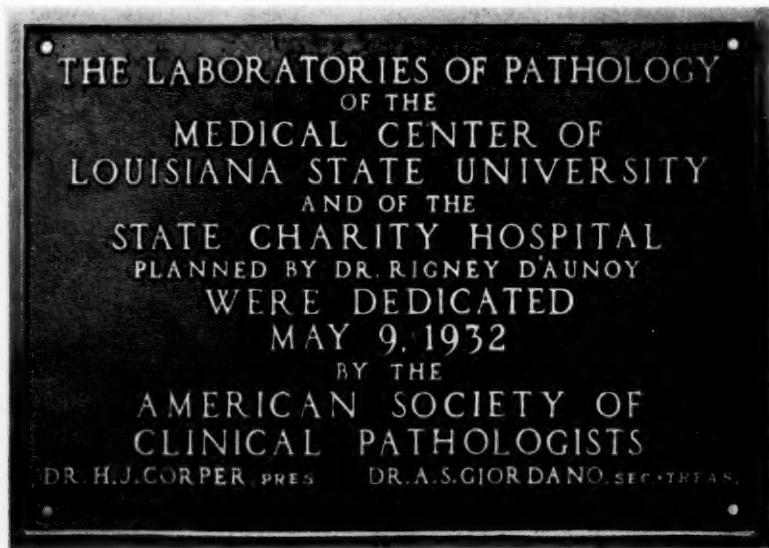
The man who first made dissecting respectable and who was the father of modern gross anatomy was Andreas Vesalius, a Belgian, who lived during the sixteenth century. By means of actual dissection of the human body he constructed for us its gross anatomy. Just as Vesalius marked an epoch establishing modern anatomy so William Harvey, the Englishman in the seventeenth century, demonstrated the circulation of the blood signalizing the epoch of the beginning of modern physiology. In the same century Malpighi founded histology and descriptive embryology demonstrating the structure of the lungs, of the kidney and spleen, and of the capillaries.

The ideas held by scientists regarding the nature of disease was in a chaotic state in the seventeenth century. One school looked upon disease as an obstruction of blood vessels, another school taught that disease was explainable in terms of acidity. Still others held to the theory of vitiated humors.

Modern pathology began with the insistence of Giovanni Battista Morgagni (1682-1771) upon thoroughness and accuracy of detail in the study of morbid anatomy. Sydenham in the seventeenth century had emphasized the importance of accurately reading the story of disease from the living sick body but Mor-

gagni in the eighteenth century stressed the importance of reading with serupulously careful, naked eyes the gruesome story from the telltale marks and lesions that disease leaves upon the bodies of those that it has slain, and attempting to correlate them with the symptoms which the patient presented during his illness.

The early nineteenth century illustrated in René Laennec the foundational value of careful and extensive studies in the death house, the postmortem room. An early intensive pathological



BRONZE TABLET PLACED IN THE DEPARTMENT OF PATHOLOGY OF THE MEDICAL CENTER OF THE LOUISIANA STATE UNIVERSITY

experience by Laennec wrought a revolutionary change of approach to the problems of diagnosis. Now a really objective examination of organs, particularly those of the chest, was made possible. By comparing stethoscopic findings on the living patient with postmortem findings, Laennec elucidated the conceptions of various diseases, and in particular tuberculosis.

At the end of the nineteenth century there appeared on the horizon a new concept, cellular pathology, introduced by the greatest pathologist the world has ever known, Rudolph Virchow

of Berlin. He maintained that the cell constituted the ultimate basis from which knowledge of both health and disease is to be derived, here the ravages and telltale marks of disease are best to be read, a lesson made possible largely by marked improvements in microscopes. The end of this century marked the death of the doctrine of spontaneous generation with the discoveries of Louis Pasteur (1822-1895) and the founding of the science of bacteriology; and parasitic diseases by prevention and treatment began to decline or disappear. The picture of death was changing.

I need hardly stress before this group the tremendous importance to the physician and mankind as a whole of exploring death and yet I cannot refrain from citing at least a few outstanding examples in modern times in which the postmortem room was invaded for material assistance and for knowledge, which you can no doubt multiply by hundreds of other examples from your own experiences.

Since this is the semicentennial year of the discovery of the tubercle bacillus and since Robert Koch is rarely referred to as a pathologist, it is appropriate that I remind you that he made frequent use of the death house for observation, study and especially for material. Those who had the pleasure of knowing Koch intimately knew that of all his studies the one concerned with tuberculosis was dearest to him and when he performed the remarkable feat of solving the etiology within a strikingly short time he resorted time and again to his pathologic experiences and to the death house for his materials. Likewise the study of Asiatic cholera in the fall of 1883 took him to the death house of the Greek Hospital in Alexandria, Egypt, where the characteristic rod was found in ten postmortem examinations and again at the Medical College Hospital at Calcutta where thirty-two postmortem examinations were performed. His therapeutic use of various tuberculin preparations again brought him intimately into the postmortem room to note the results of the use of these agents.

You will remember also that Herman Brehmer of Germany, the founder of the first successful sanatorium for tuberculosis in the world and today the largest private sanatorium in existence, received his inspiration for treating tuberculosis from the postmor-

tem table. He noted in persons dying from consumption that some of the solitary lesions in the lungs had healed and from these observations wrote a thesis on the "Curability of Consumption" and initiated his life work.

Probably the most outstanding confession of the significance of pathology and the postmortem examination is recorded by our own William Osler. Last year the members of our Society had the pleasure of visiting the little old brick building in which he performed his autopsies in Philadelphia and were able to examine his original protocols. Starting with an interest for pathology he is found in Berlin in 1873 studying under Virchow who was then fifty-two years old and a master of pathologic detail. He avidly absorbed from the lessons of a genius and early in 1876 became pathologist to the smallpox hospital in Montreal, a position created for him. Here he laid the foundation for his career as a great clinician. Dr. Abbott tells us "The fact is not so well known, that during these years, and even earlier, in his student days, he was not only a pathologist, but also, essentially, and to a remarkable extent, a museum collector."

"Viewed in the light of these records, these specimens undoubtedly present, in visible and tangible form, the first stepping stones in a great career." "His practice of medicine is literally built up out of his rich memories of these and similar cases." His serious studies as a morbid anatomist continued for thirteen years until he went to Johns Hopkins.

Some of the characteristics of this profound student of morbid anatomy are well illustrated by a story told by his cousin, Marian Osborne.

"One day we were walking down the street together. He found it difficult to walk in the accepted sense of the term; his nature seemed too buoyant to allow him to place one foot before the other, as done by the more humdrum individuals. His was the true *joie de vivre* that never left him in spite of work and sorrow and years. On this day we were dancing along St. Catherine Street hand in hand, when an old and very seedy-looking man accosted us and asked for money. Uncle Bill looked at him with his penetrating brown eyes and said with a laugh, 'You old rascal, why should I give you money to drink yourself to death?' 'Well, Sir, it lightens the road going.' 'There is only one thing of value about you, and that is your hobnailed liver.' 'I'll give it to you, Sir. I'll give it to

you.' Dr. Osler laughed and putting his hand in his pocket, drew out some silver which he gave to the old man saying, 'Now, Jehosaphat, promise me you will get some soup before you start in on the gin.' The old fellow eagerly agreed and went away with infirmity in his step. The doctor looked after him with a thoughtful expression. 'Pretty cold for that poor fellow,' he murmured and then I found we were running after the beggar. 'Here, take this. I have a father of my own', said Osler, pulling off his overcoat and putting it on the astonished old man; 'you may drink yourself to death and undoubtedly will, but I cannot let you freeze to death.' 'Tell me your name, Sir?' 'William Osler, and don't forget to leave me that liver.'

"Virtue was rewarded two weeks later. The old man before he died in the hospital made his last will and testament, leaving his hobnailed liver and his overcoat to his good friend, William Osler."

It was at the Dead House at Blockley, with a crowd of students about him, that he was most constantly to be found. The Blockley Hospital, originally the Philadelphia Alms House, is the oldest hospital in the United States. In 1870 the University of Pennsylvania moved to property adjoining the Blockley Hospital. One could leave the University Hospital by the rear and enter the Blockley enclosure by a "postmortem gate" in the old wall. Near this gate stood a little red building, a half way house to the Potter's Field. The opportunity for postmortem studies was unusually good and here in spite of every inconvenience Osler was found. It might also interest you to know that Osler's book of medicine, a pathologists' book, attracted the attention of Mr. Rockefeller and led to the creation of Rockefeller Institute and finally to the incalculable benefit to humanity which the General Education Board has rendered with Mr. Rockefeller's money. A gift inspired by a lover of morbid anatomy, and an explorer in this field.

At Hopkins Osler always first brought visitors to the Pathological Laboratory and to "Popsy" Welch. What can better express his estimation of this branch of medical sciences!

To return to the dynamic phases of death, before closing permit me to use Peyton Rous' recent theme the modern dance of death depicted in his excellent scientific volume of that name, and to again refer to Warthin's Pathology of the Aging Process. Both feel that we are quantitatively and gradually approaching the ideal

pinnacle of the maximal century age, the biologic span of life, and that the main changes wrought by medical science have been and will be concerned with the effects upon pathologic extrinsic death and pathologic intrinsic death. Accordingly old age is inevitable and escape is only possible for those who meet premature pathologic death. They are not inclined to favor the view that old age can be deferred nor that rejuvenescence may some day be possible. Be that as it may, some just as worthy minds may contest this and remind us again of what Duclaux once said "It is because science is sure of nothing that it is always advancing." Need we cite more than electrons, the Hertzian waves and cosmic rays to awake the spirit of working on to push back the boundaries of the unknown. It is well here to remember Minot's words: "We have enthroned science in the imagination, but we have crowned her with modesty, for she is at once the reality of human power and the personification of human fallibility."

Even though we agree with Rous that the underlying actions and reactions to disease within the body remain essentially what they were in the past the pathologist will have to be on the alert to discover the early and so-called new manifestations of previously undescribed disease conditions as well as to discern the quantitative or relative significance of diseases in certain communities and at certain times. Painstaking and careful observations are as essential today as they were in Vesalius' time, or as they were for Osler.

In Utopia the necessity for the pathologist and for exploring death may disappear and I dedicate this Department of Pathology of the Medical Center of Louisiana State University and of the State Charity Hospital to a useful and valuable service for preserving the health and life of mankind in this community and in setting a good example for others to follow.

LYMPHOMATOUS COMPRESSION OF THE SPINAL CORD*

WITH A CASE REPORT OF THE HODGKIN'S TYPE

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If syphilis be accepted as the most protean of infectious diseases, certainly, that group of neoplasms falling into the general classification of lymphoma, should take first rank in its protean aspects among the neoplastic diseases.

Unfortunately, there is no generally accepted terminology for the conditions under consideration. Etiology is, to some extent, a matter of opinion, and pathogenesis is obscure. Indeed, confusion may arise in the interpretation of histologic changes observed in the same specimen of lymphatic tissue. Nevertheless, at least two factors are common to the group; first, a progressive enlargement of fixed or disseminated lymphatic tissue; and second, a fatal termination.

Lymphomatous nodes have certain characteristics. Growing expansively, the early nodules are firm, discrete, freely movable, and not tender, so long as there is no accompanying periadenitis. In older nodes, the value of these factors for differential diagnosis is relatively more limited, so that biopsy and microscopic examination are necessary to establish their identity. Frequently their gross appearances will distinguish lymphomatous nodes from those of acute and chronic infectious origin, and to some extent, the cut surfaces of the various types of lymphomas are suggestive, although seldom diagnostic.

As a rule, however, these various observations are of relative importance only and resort must be had to microscopic study.

* Read before the Eleventh Annual Convention of the American Society of Clinical Pathologists, New Orleans, Louisiana, May 6-9, 1932.

Since the histology of the lymphomatous node is relatively limited, usually it is not difficult to agree on the presence or absence of cytologic elements. It is their interpretation, classification, and terminology, which become diagnostic stumbling blocks. As a rule, the less complicated the terminology, the less confusion will arise; therefore, the simplest classification sufficiently comprehensive has the merit of being the most useful for practical purposes.

Owing to its simplicity and adaptable character, the writer has adopted, with slight modification, the classification followed by Baldridge and Awe.² While it is not my object to discuss or defend the terminology of any classification, it is desirable, because of the existing confusion, to avoid the risk of being misunderstood.

The grouping referred to, in an analysis of 150 lymphomas, is based chiefly on the cytologic criteria; and, with the more common synonyms in brackets, is outlined as follows:

Lymphoma (Lymphoblastoma) (Malignant lymphoma)

- (1) Sclerosing type (Hodgkin's disease) (Lymphogranuloma)
Fibrosis, mononuclear and multinuclear giant-cells (Dorothy Reed), lymphocytes, eosinophiles in a connective tissue reticulum, with or without necrosis.
- (2) Endothelial type (Lympho-epithelioma)
Uncommon; epithelial (or endothelial) groups of cells with poorly staining cytoplasm resembling a syncytium; may be mistaken for metastatic carcinoma.
- (3) Lymphoblastic type (Lymphosarcoma)
Cells resembling the maternal cells of a normal germinal center are found throughout the node; moderate amount of stroma; tendency to invade outside the capsule.
- (4) Lymphocytic type
 - (a) With leukemia (Lymphatic leukemia)
 - (b) Without leukemia (Aleukemic leukemia)

Uniform small lymphocytes without stroma; a few trabeculae; looks like a sac (capsule) full of cells.

Comparatively, the histologic variations in these types of lymphoma are no greater than the architectural variations in carcinoma of the breast.

All reference to the sites of predilection of lymphomatous neoplasia has been studiously avoided up to this point, lest the importance of this factor be somewhat overshadowed. The widely disseminated and protean character of lymphomatous disease, especially the Hodgkin's type, has not been accorded due emphasis in medical texts and monographs. Ginsburg,⁹ in commenting on this anomaly, cited the example in Elsberg's recent monograph on "Tumors of the Spinal Cord." In this excellent work there is no reference to a Hodgkin's involvement of the cord; yet, in one New York hospital (Montefiore) alone Ginsburg stated that, of thirty-six patients with Hodgkin's disease from 1914 to 1925, ten were observed in which "definite invasion of the nervous system was a striking clinical phenomenon." Davidson and Michaels⁶ made a similar allusion in reporting twenty-six cases of lymphosarcoma from the same institution between 1922 and 1929, four of which showed signs of spinal cord compression. In spite of numerous isolated communications attesting to the varied manifestations of lymphomas, it is a fact that these data have not been adequately assimilated into our standard literature. There is still a widespread belief that lymphomata are mere "affections of the absorbent lymph nodes and spleen."

Although it is unquestionable that in a large series of cases, the peripheral lymph nodes are the first to attract clinical attention, there are impressive and diverse groups of outstanding presenting symptoms of disease due to lymphoid neoplasia, with but slight, if any, involvement of the peripheral glands. Leukemic infiltrations in lymphatic leukemia have, as a group, been anticipated, yet there are instances where the typical leukemic blood picture has been delayed (aleukemic states) until long after the onset symptoms of dissemination. Failure to associate such symptoms and a typical blood picture is quite inexcusable. In the sclerosing, endothelial, and lymphoblastic types of lymphoma no trustworthy aid is offered by the blood picture. The key to diagnosis will rest with the finding of a lymph node for sectioning, if perchance one be available.

That the involvement of various organs and tissues, other than

the lymphatic system, is not limited merely to a few isolated foci of academic interest and inconsequential nature, has been stressed by many authors. The crux of the matter is, that the importance of metastases and disseminations (in lymphomas) frequently transcends that of the primary pathology. The resulting clinical symptoms and signs are so diverse and manifold that, were the condition more common, it would be a problem of the first magnitude in differential diagnosis.

By way of illustration, a few of the chief and outstanding symptoms for which patients have sought relief have been: generalized and localized pruritus, paroxysmal tachycardia, melena, diarrhea, jaundice, hematuria, motor paralysis, sensory disturbances, and intermittent fever. From the diversified list compiled of other conditions simulated, one finds: malta fever, hypernephroma, bronchial asthma, pulmonary tuberculosis, osteomyelitis, and gastric carcinoma. Of the organs and systems chiefly affected, the skin, nose, tonsils, trachea, and bronchi, stomach, intestine, liver, spleen, pancreas, osseous, nervous, and hematopoietic systems. Indeed, there is evidence to show that in the sclerosing type alone, no organ or tissue has escaped invasion.²

In a general way, the type and mechanism of dissemination may be summed up as follows: In the sclerosing and endothelial types of lymphoma, the disseminated lesions are of the nature of discrete nodules for which the term metastasis is perhaps more appropriate. In the lymphoblastic type, the lesions are not only discrete, but there is likely to be a localized destruction of the parenchyma, or an infiltration in which the lymphoid cells tend to be arranged in rows between the parenchymatous elements or surrounding the vessels. In the lymphatic type, both with and without leukemia, there are perivascular and intercellular infiltrations of lymphoid cells, but rarely any nodule formation. In long standing cases, the leukemic infiltration becomes almost universal. In general, it may be said, the younger the patient the more virulent the disease; in childhood, rapidly fatal, whereas in adults it is essentially chronic.

The invasion of the nervous system was first reported by Murdoch¹⁴ in 1870, and has since been noted by Goodhart,¹⁰ Gowers,¹¹

Longcope,¹³ Askanazy,¹ Dietrich,⁶ Welch,²¹ Sternberg,¹⁸ Weber,²⁰ Pancoast,¹⁵ and others. That clinical symptoms resulting from such invasion may dominate the clinical picture is attested to by the case reports of Askanazy, Hecher and Tischer¹² Walthard¹⁹ and Reese and Middleton.¹⁷

In 1917, Bassoe³ collected and published the reports of cases of paraplegia proved at necropsy to have been caused by leukemic infiltration or chloroma in the spinal cord. He believed that a number of these cases have been described under that disease of the hematopoietic system known as chloroma, a condition admittedly having all the features of leukemic infiltrations with the unexplained characteristic of a grass-green color of the tissue which soon changes when exposed to the air. Green tumors are said to have been found in all forms of acute and chronic leukemia of both myelogenous and lymphatic types. Von Recklinghausen, in 1885, called attention to the similarity of the histologic picture of leukemia and chloroma, and in 1893, George Dock⁷ in analyzing seventeen cases then known, including one of his own, was fully aware of the leukemic nature of chloroma.

Dock and Warthin,⁸ in 1904, reviewed twenty-two cases, in nine of which there was involvement of the vertebrae "usually in the periosteum of the bodies, sometimes in the dura, sometimes in the fat of the vertebral canal" with atrophy and degeneration of the cord.

Among the authors of the more widely used text-books are Ewing, Aschoff, McCallum, and Negeli who divided chloroma into lymphoid and myeloid types. Mallory alone considered it a part of myelogenous leukemia. Using the oxydase reaction in blood smears as a criterion for classification, Brannan¹⁶ was of the opinion that chloroma should be considered a subvariety of myelogenous leukemia only.

From a review of the literature, Reese and Middleton concluded that the most common form of paraplegia in leukemia is due to epidural or peridural infiltration with resultant mechanical compression of the cord. Intramedullary areas of softening due to leukemic infiltrations occluding spinal cord vessels have rarely been reported.⁴

In the sclerosing type of lymphoma, peridural infiltration resulting in a collar-like mass partially encircling the cord, with compression as a sequel, seems to be much more common than intramedullary invasion. It is of interest to note also the frequency with which that portion of the spinal canal between the third and eighth dorsal vertebrae has been involved, altho true to form, lymphomatous infiltrations in the central nervous system seem to scorn any particular site of predilection: no portion is immune. Curiously, in the case of myelogenous infiltration of the spinal canal reported by Basal in 1917, the "region of the 4th, 5th, and 6th thoracic vertebrae" were found involved.

The lymphocytic and sclerosing types of lymphoma, while relatively more frequent than the endothelial and lymphoblastic types, seem to be the chief invaders of the nervous system. Baldridge and Awe reported but one involvement by the combined endothelial and lymphoblastic type in their analysis of 150 consecutive cases as against nine for the sclerosing type, and five for the lymphocytic type. Davison and Michaels reported four cases of lymphosarcoma with signs of cord compression.

As to the clinical recognition of lymphomatous compression of the cord, perhaps the most comprehensive deduction is to include this possibility in the differential diagnosis in appropriate conditions. Cord symptoms associated with a leukemic blood picture form a combination almost equivalent to diagnosis; however a temporary aleukemic state must be kept in mind. Cord symptoms in a patient with lymphomatous nodes, especially of the sclerosing (Hodgkin's) type may be strongly suspected to be due to a lymphomatous peridural color. In case neither a leukemic blood picture exists nor a lymph node is available for section, reliance must be placed chiefly on clinical symptoms alone.

Reese and Middleton felt that the syndrome of paraplegia occurring in children and young adults, with pain in the back, radiating intermittently into the legs, followed by a spastic paretic gait, later by bladder retention and rectal incontinence and finally by a complete compression syndrome associated with trophic disturbances, serves to differentiate leukemic compression from that due to other tumors. This sequence of events was remarkably closely followed in the case I wish to record.

CASE REPORT

R. K., a tenth-grade school boy, aged fifteen years, was the son of a colleague. With his father, I first examined this patient February 23, 1931. The chief symptoms at that time were nearly complete motor paralysis and numbness in both legs. The present illness dated from December, 1930, when the patient complained that his feet and legs felt heavy. On one occasion he fell down several steps without injury. His father observed that the boy's muscular movements seemed clumsy at times; also, that he frequently shifted his posture from erect to stooping and from side to side. He complained of pain in the back between the scapulae.

Following a common cold January 26, 1931, which lasted two days, the patient gradually lost weight and color, and developed an obstinate constipation, necessitating manual relief. February 21, 1931, motor weakness in both legs developed rapidly, and February 23, he last walked alone.

The patient was referred to Dr. C. Van Epps at Iowa City, to whom I am indebted for a record of the balance of the history and clinical examination. By February 26, motor paralysis in the legs had become complete, and numbness extended to the costal margins. An increasing slowness in urination culminated in the necessity for frequent catheterization after March 1st.

Examination by Dr. Van Epps, March 2, 1931 revealed: weight 141 pounds, a loss of 13 pounds in one month; height 70 inches; marked motor and sensory loss up to the midepigastrium; paralysis of the spastic type involving both legs, with increased deep tendon reflexes, bilateral ankle clonus, and bilateral plantar extension. Clinical impression: meningo-myelitis with compression at seventh thoracic segment.

Laboratory data; Blood: erythrocytes, 4,850,000; leukocytes, 8,900 to 11,200; hemoglobin 90 per cent (Dare); neutrophiles, 73 per cent; eosinophiles, 2 per cent, lymphocytes, 20 per cent; unclassified, 5 per cent. Wassermann negative. Cerebrospinal fluid: Pressure, 2 mm. Hg. increased slowly to 6 mm. Hg. on coughing; fluid, clear; 3 lymphocytes per cu. mm.; globulin, 151.9 mgm. per cent; sugar, 79.0 mgm. per cent. Cerebrospinal fluid, March 5: Pressure, 8 mm. Hg. increased slowly to 14 mm. Hg. on coughing; eight lymphocytes per cu. mm.; globulin, 183.0 mgm. per cent; sugar, 78.0 mgm. per cent. X-ray of spine: anterior-posterior and lateral views: Negative.

March 19, 1931, a laminectomy by Dr. H. Bye extending from the third to the eighth thoracic vertebrae, revealed a thickened compact area of peridural fat at the fifth vertebrae, also an extradural collar-like mass, approximately 10 cm. wide, compressing the cord. Microscopic examination of tissue from the spinal canal by Dr. Hansmann, showed "small round cells with oval and round deeply staining nuclei, and a narrow rim of bluish pink cytoplasm; also large epithelial cells with paler staining nuclei; a small number of Reed giant cells, and a relatively large number of eosinophiles; and a well marked diffuse fibroblastic reaction."

The patient's recovery from the operation was uneventful, but without symptomatic relief. He suffered intensely painful spasmoidic contractions of both lower limbs, resulting finally in flexed contractures and marked atrophy. He became very anemic, and emaciated, expiring January 7, 1932 from complete exhaustion, and a terminal infection.

AUTOPSY REPORT

Male body, greatly emaciated. The thighs are flexed on the body, and the legs on the thighs at angles of about 45°, so that as the body lies on the side, it forms the letter Z. The joints are not ankylosed; both legs and thighs can be extended with sufficiently great pressure as to overcome the contractures. Articulations of the upper extremities are normal; there is no rigor.

The skin is white, of fine texture, and the mucosae are very pale. There is a moderate oedema of the feet, ankles, and in the lumbar region. The hair of the head is black and dry, the body hair is scanty. The external genitalia and rectum are normal. The external auditory and nasal passages are negative. The eyelids are approximated, the sclerae are pale, the pupils are dilated, equal and regular. There are no palpable lymph nodes. There are no external anomalies. Aside from the adduction and acute flexion of the thighs and legs, there are no deformities. There is a 25 cm. healed surgical incision extending down the mid-line of the back, beginning 5 cm. below the spine of the seventh cervical vertebra.

On median section, the subcutaneous tissues are oedematous; the panniculus is very thin and orange colored. The blood is thin and dark. The abdomen contains no free gas or liquid. The urinary bladder is distended with ammoniacal urine; the walls are thin and parchment-like. Other abdominal pelvic viscera are of normal size and position.

The costal cartilages cut easily. The marrow of the sternum is light red and granular. There is approximately 1300 c.c. of blood stained fluid in each thorax. The mediastinum contains large irregular masses of lymphatic nodes arranged roughly in three groups.

(1) In the region of the thymus remnants, there are from twenty to thirty nodes, some discrete, some coalesced and matted together, varying from pea to hickory-nut size. These nodes and the inter glandular connective tissue are very adherent to the larger vessels, so that it is almost impossible to dissect the mass free without cutting into vessel walls.

(2) A second group is composed of one large rhomboidal mass occupying the space between the sternum and vertebrae, adherent to the right and posterior aspects of the trachea. Grossly, this mass consists of one large node anteriorly and a fringe, and small coalesced nodes posteriorly.

(3) A third group of nodes is in the bifurcation of the trachea, and extends both right and left surrounding the bronchi. There are approximately fifteen of these nodes, some of which are coalesced, others discrete, and all very adherent to the trachea, bronchi, and perivertebral tissue posteriorly. On section grossly,

the smaller nodes in each group present a smooth, pink, shiny surface. The medium sized and larger nodes show more or less patchy fibrosis. The largest nodes in the bronchi region show extensive fibrosis with streaks and trabecula of lymphoid tissue. This group of nodes is also adherent to the ventral surface of the perivertebral fascia, particularly over the body of the fifth thoracic vertebra, but also over-lapping the fourth and sixth vertebrae. Removal of the glandular mass from the vertebral bodies reveals marked changes in the bodies of the fourth, fifth, and sixth vertebrae. The body of the fifth is much softer, and more granular. The bodies of the fourth and sixth are distinctly softer and more granular than normal, also less so than the body of the fifth. These bones may be cut into with a knife, and on section show numerous pinhead to pea-sized masses of a light pale green, jelly-like substance. Within the spinal canal and around the dura there is a band-like mass of firm light green colored material nearly completely encircling the dura. It is as though a ring had been cut and spread apart slightly. The spinal cord is distinctly compressed to about one-half size within the dura directly beneath this band. The band varies from 4 to 6 cm. in width. On each side of the band, both up and down the dura for a distance of 5 or 6 cm., there are pea-sized masses of this same soft light green material. Peridural compression of the spinal cord is very clearly demonstrated.

The pericardium contains about 25 c.c. of clear straw colored fluid. The heart is a little larger than the cadaver's fist. The muscle is pale. The valve flaps and orifices are of normal size. There are no vegetations nor deformities. The endocardium is smooth and pale.

The lungs are air containing throughout. The hylum of each lung shows several large peribronchial lymph nodes, having the same appearance of those in the mediastinum. The larger nodes contain much more connective tissue than the smaller.

The liver is of normal size and consistency; the cut surface has a fatty sheen. The bile passages are free of exudate and concretions. Serial sections show no gross changes, other than the obvious fatty change. A careful search for nodules revealed none.

The spleen is of normal size, a little softer than normal; on section dark red and moist. There are no gross changes, no nodules.

Both kidneys show multiple pinhead sized masses on the surface, yellowish white in color. The pelves of the kidneys and ureters are patent, not dilated, and have a moist shiny surface.

The adrenals are of normal size, and show no gross changes. The retroperitoneal hemolymph nodes appear normal in size and on section.

The gastro-intestinal tract shows no gross change aside from the pale color, which is everywhere apparent throughout the peritoneum.

The urinary bladder is distended and contains about 1000 c.c. of ammoniacal turbid urine. The wall is parchment-like. Both testes are in the scrotum, and appear normal on section.

The bone marrow from a rib is light red, cellular, and less moist than normal.

MICROSCOPIC REPORT

Mediastinal lymph nodes: The smaller nodes reveal a fine fibrillar reticulum forming a ground substance for lymphocytes, eosinophiles, mononuclear and multinuclear giant-cells. The larger nodes are less cellular, the connective tissue reticulum is much more dense and abundant, and the normal architecture of the node is rearranged—completely lacking in the larger nodes.

Liver: The cells are pale staining, and have a ground glass appearance. The cords are somewhat broken up. The walls of the veins are thickened. The sinusoidal spaces contain a few polynuclear cells and larger cells containing a brown pigment (mononuclears).

Spleen: Histologically, the spleen is well preserved. The splenic arteries contain groups of cells of the lymphocytic type, while the veins are distended with red cells. There is a moderate cellular hyperplasia, and an occasional field showing eosinophiles.

Bone Marrow: There is a very fine, delicate reticulum. The cells constituting the mass of the marrow are vesicular, with pale-staining nuclei, and a small rim of protoplasm. There are less than the normal number of erythrocytes, none of which are identified as containing nuclei. The capillary and sinusoidal network form irregular cords and groups.

Sections from the fifth thoracic vertebra show an enlargement of the marrow spaces and a rarefaction of the bony trabeculae. The spaces are filled with cells resembling the large lymphocytes. There are a few cells having eosinophilic granules. There is a delicate fibrillar network more marked in some fields than in others. It is apparent that cords and groups of these cells resembling large lymphocytes extend from the marrow spaces of the vertebral body through the periosteum, through the prevertebral fat and fascia, and into the spinal canal. By direct extension through the lymphatics, blood vessels, and between the bony trabeculae there is a continuity of the bone marrow new-growth and the collar-like peridural mass. Histologically the structure is the same.

SUMMARY

- (1) Attention is called to the protean character of the manifestations of lymphomatous neoplasia.
- (2) A case of lymphomatous compression of the spinal cord is reported with clinical, laboratory, and histologic data.
- (3) The principal pathologic changes noted are:
 - (a) Three groups of greatly enlarged, coalesced lymph nodes, very adherent to surrounding structures in the mediastinum, around and behind the bronchi.
 - (b) The adhesion of the lymph nodes to the prevertebral fascia.

- (c) The marked softening and granular appearance of the bodies of the fourth, fifth and sixth vertebrae, and the presence therein of a light pale green gelatinous substance.
- (d) Compression of the spinal cord by a collar-like mass most marked at the level of the 5th thoracic vertebrae.
- (e) Continuity of cellular changes within the vertebral bodies, and the peridural collar-like mass.

REFERENCES

- (1) ASKANAZY, N.: Lymphogranulom des Knochenmarks. Verhandl. d. deutsch. path. Gesellsch., **18**: 78-83. 1921.
- (2) BALDRIDGE, C. W., AND AWE, C. D.: Lymphoma. Arch. Int. Med., **45**: 161-190. 1930.
- (3) BASSOE, PETER: Leukemic infiltration in the spinal canal as a cause of paraplegia. Jour. Nerv. & Ment. Dis., **47**: 180-190. 1918.
- (4) BAUDOUIN, A., AND PARTURIER, G.: Sur les complications nerveuses des leucémies. Rev. Neurol., **18**: 673-680. 1910.
- (5) DAVISON, C., AND MICHAELS, J. J.: Lymphosarcoma. Arch. Int. Med., **45**: 908-925. 1930.
- (6) DIETRICH: Discussion of Askanazy's paper. Verhandl. d. deutsch. path. Gesellsch., **18**: 83. 1921.
- (7) DOCK, GEORGE: Chloroma and its relation to leukemia. Am. Jour. Med. Sci., **106**: 152-185. 1893.
- (8) DOCK, GEORGE, AND WARTHIN, A. S.: A new case of chloroma with leukemia. Tr. Assn. Am. Phys., **19**: 64-115. 1904.
- (9) GINSBURG, S.: Hodgkin's disease. Arch. Int. Med., **39**: 571-595. 1927.
- (10) GOODHART, J. F.: A case of Hodgkin's disease. Tr. Clin. Soc. London., **10**: 57-63. 1877.
- (11) GOWERS, W. R.: Hodgkin's disease. In: REYNOLD'S, J. R.: A system of medicine. London, McMillian & Co., **5**: 306-367. 1879.
- (12) HECKER, H. V., AND FISCHER, W.: Zur Kenntnis der Lymphogranulomatose. Deutsche med. Wehnsehr., **48**: 482-484. 1922.
- (13) LONGSCOPE, W. T., AND McALPIN, K. K.: Hodgkin's disease. In: Oxford Medicine. New York, Oxford University Press, **4**: part 1, 1-43. 1921.
- (14) MURCHISON, C.: Case of "lymphadenoma" of the lymphatic system, spleen, liver, lungs, heart, diaphragm, dura mater, etc. Tr. Path. Soc., London, **21**: 372-383. 1870.
- (15) PANCOAST, H. K., AND PENDERGRASS, E. P.: The occurrence of herpes zoster in Hodgkin's diseases. Am. Jour. Med. Sci., **168**: 326-334. 1924.

- (16) REDDICK, W. G., AND BRANDES, W. W.: Chloroma. Its relation to the leukemic and neoplastic processes. *Texas State Jour. Med.*, **27**: 297-300. 1931.
- (17) REESE, H. H., AND MIDDLETON, W. S.: Mechanical compression of the spinal cord in tumorous leukemic infiltration. *Jour. Amer. Med. Assn.*, **98**: 212-217. 1932.
- (18) STERNBERG, C.: Lymphogranulomatosis. *Klin. Wehnschr.*, **4**: 529-534. 1925.
- (19) WALTHARD, K. M.: Rückenmarkserweichung bei Lymphogranulom in im extraduralen spinalen Raum; Lymphogranulom des Uterus als Nebenbefund. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, **97**: 1-19. 1925.
- (20) WEBER, F. P.: Lymphogranulomatosis maligna (Hodgkin's disease) with recurrent purpura and hemorrhagic symptoms. *Proc. Roy. Soc. Med.*, **17**: 7-10. 1924.
- (21) WELCH, J. E.: A tumor of the neck showing unusual histological features. *Proc. New York Path. Soc.*, **10**: 161-169. 1910.

"SENSITIVITY" TO SULPHYDRYL

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In a report⁶ on the use of compounds of the sulphhydryl group, in particular, thioeresol in wound healing, it was stated that after a short time of application a certain number of individuals, so treated, developed an itching rash. It was found during the course of application of sulphhydryl compounds (thioeresol, benzyl mercaptan) to the skins of rats and mice that a certain percentage of these animals also reacted in a similar way.

In order to obtain an estimate of the number of humans who reacted to this group, 450 "normal" individuals, 290 women and 160 men, aged from eighteen to seventy years, were subjected to the following procedure. In addition, for reasons given below, thirty-two women and forty-five men with known carcinoma were also tested.

The skin in the cubital space of the elbow was chosen for convenience. Thioeresol in 1 per cent alcohol solution was painted once on the right arm; the same concentration of cresol in the same solvent was used on the left arm. Observations were made for a period of three weeks.

RESULTS

Eighteen of the "normal" individuals, ten women and eight men, were found to react, (see fig. 1). Among the individuals with carcinoma, five reacted. The time of appearance of the reaction varied from three to eighteen days. None reacted to cresol.

In those who did not react, no sensation whatever was noted and no objective findings could be discovered. The first symptom in those who did react was an itching, which grew in intensity until it equaled that of well developed ivy poisoning, (to use the comparison of one of the individuals). A diffuse redness was

sharply limited to the area which had been painted, small papules and blisters appeared and itched intensely. The blisters perhaps coalesced and, in extreme cases, produced a very large bleb. The erythema disappeared from between the papules and vesicles, allowing them to become more prominent, they then gradually dried, a few crusts were formed, and finally the whole area showed mild desquamation. Red to brown pigmentation remained for several weeks more.

DISCUSSION

The facts that certain chemical groups in complex molecules elicit response in different individuals, and that rearrangement



FIG. 1. REACTION ON ARM OF NURSE TWO WEEKS AFTER APPLICATION
Nothing appeared on the control arm

of these groups in the molecules changes the conditions, have been brought out especially in the studies of Landsteiner and associates.³ That this is an example of such a phenomenon seems quite probable.

Since the thesis that the sulphhydryl group is essential to cell division is now established,¹ the first thought is that those individuals who are sensitive to this group are also sensitive to cell division phenomena. It will require time and much more data to discover this relation, if present.

The eruption on the skins of the rats and mice which exhibited

this reaction disappeared in about two weeks, in spite of continued painting with the material. The same end result was found at the end of about six weeks as in the animals not "sensitive," namely, thickened skins, with no histological differences.^{2, 4, 7}

Five patients with local ulcerating surfaces were treated with the 1:10,000 watery suspension of thiocresol to promote healing.⁵ The original carcinomas had been treated by wide surgical excision in three and by cautery in two. In all five the eruption appeared around the edges of the wounds after about a week and treatment was discontinued. These also were the patients with carcinoma who reacted positively as noted. We have not treated any other patients with wounds left from removal of carcinomas.

An observation recorded in two young women is to the effect that after intervals of three and two weeks respectively, after painting, with no sign of reaction, the eruption appeared suddenly on the first day of menstruation.

SUMMARY

Local areas of the skin of the arms of 450 "normal" individuals were painted once with 1 per cent alcoholic solution of thiocresol and controlled on the other arm with one per cent cresol solution. Of these, eighteen individuals reacted by the production of an itching rash.

Among seventy-seven individuals with carcinomas similarly treated, 5 reacted, all of whom had open, ulcerating wounds; the region around the wound also reacted.

The special significance of this "sensitivity" to the sulphydryl group is unknown, but it is probably related to the general phenomena of sensitivity to specific chemical groups and arrangements. Since the use of the sulphydryl group in p-thiocresol and other compounds is increasing, attention is drawn to this phenomenon. At present it is recommended that in those who react, treatment be discontinued.* It is also recommended

* Bogert and Husted (Jour. Pharm. & Exp. Therap., **45**: 189-207. 1932), call attention again to the susceptibility of certain individuals to the benzothiazoles in which similar latent periods are described as were encountered with -SH compounds.

that such treatment of ulcerating carcinomatous wounds be avoided in this group.

REFERENCES

- (1) HAMMETT, FREDERICK S.: The natural chemical equilibrium regulative of growth by increase in cell number. *Protoplasma*, **11**: 382-411. 1930.
- (2) HAMMETT, F. S.: The proliferative reaction of the skin to sulphydryl and its biological significance. *Protoplasma*, **13**: 331-347. 1931.
- (3) LANDSTEINER, K., AND LEVINE, P.: Experiments on anaphylaxis to azo-proteins. *Jour. Exp. Med.*, **52**: 347-359. 1930.
- (4) REIMANN, STANLEY P.: Proliferation of rat and mouse epithelium from sulphydryl. *Protoplasma*, **10**: 82-83. 1930.
- (5) REIMANN, STANLEY P.: Use and reasons for the use of thiocresol to stimulate wound healing. *Jour. Am. Med. Assn.*, **94**: 1369-1371. 1930.
- (6) REIMANN, STANLEY P.: Thiocresol in wound healing. *Ann. Surg.*, **93**: 624-627. 1931.
- (7) REIMANN, STANLEY P.: The hyperplastic reaction of the skin to sulphydryl and its significance in neoplasia. *Am. Jour. Cancer*, **15**: 2149-2167. 1931.

EDITORIAL

LABORATORY SERVICE FOR HOSPITAL RESIDENTS

Not the least vexing of the many problems confronting the average hospital is the arrangement of the Resident's service in the clinical laboratory so that the experience may be of practical value in his later career.

This difficulty arises in part from the fact that the allotted period is usually too short to permit either thorough training or a comprehensive survey of this phase of the practice of medicine; and in part from the frequent tendency of Residents to regard this phase of their hospital career as a period of true servitude, indeed. Lacking in the drama of the accident service, and the operating room, impersonal in the material with which its work is concerned, the laboratory service is often of importance to the Resident only as standing between him and these promised lands; something to be encountered with resignation and encompassed with relief.

The laboratory is not always absolved of responsibility for the perpetuation, if not the development, of this attitude. Lacking the skill and experience for the more specialized laboratory procedures, too often the Resident spends his time in the examination of urine and the counting of blood cells, procedures important enough in themselves but likely to become monotonous when confronted in bulk. And it may be questioned with justice if the return to the Resident is commensurate with the time expended and if such a plan best achieves the purpose in view. It cannot be too strongly emphasized that the purpose of such a laboratory service is not the acquisition of technical skill.

While it is impossible in these modern days to escape utilization of the resources of the laboratory, it is also inevitable that these are best applied through the medium of those skilled in their use.

In the last analysis the profitable utilization of the laboratory in the study of disease depends not so much upon an extensive

knowledge of technic but upon a thorough appreciation of what to do, when it may be most profitably done, and above all, what is its clinical significance and utility when it has been done.

In other words, it is not the test but its clinical significance and application which is of paramount importance, and emphasis of this fact should be the primary purpose of the Resident's laboratory service.

Technical facility cannot, however, be entirely neglected; some things the Resident must be taught; first, those which he may be called upon to perform as emergencies in the absence of the regular laboratory force and, second, those which, in the early days of his professional career, he may well utilize in the routine examination of the patient.

It is of great practical value, also, that in the collection of specimens for varied purposes, he acquire a thorough and relatively extensive training in venipuncture, both because the necessity will inevitably, and even frequently, arise in the course of clinical practice, and because lack of skill in this simple procedure transforms it into a gory performance regarded with apprehension by the patient, attended by unpleasant and, perhaps, even serious sequelae, reflected in an unfavorable, even though unjustified, estimate of the ability of the physician.

Every endeavor, therefore, should be made to present the laboratory service to the Resident in this light. He should learn by observation the proper methods for the collection and forwarding of laboratory specimens, for the success or failure of laboratory examinations, the reliability of their results, and consequently their significance may often be directly dependent upon the proper collection and after treatment of the specimen.

He should learn, by observation, reading, and discussion how to select from the multiplicity of available procedures those most likely to be informative in a given case and, particularly, how to interpret and utilize their results.

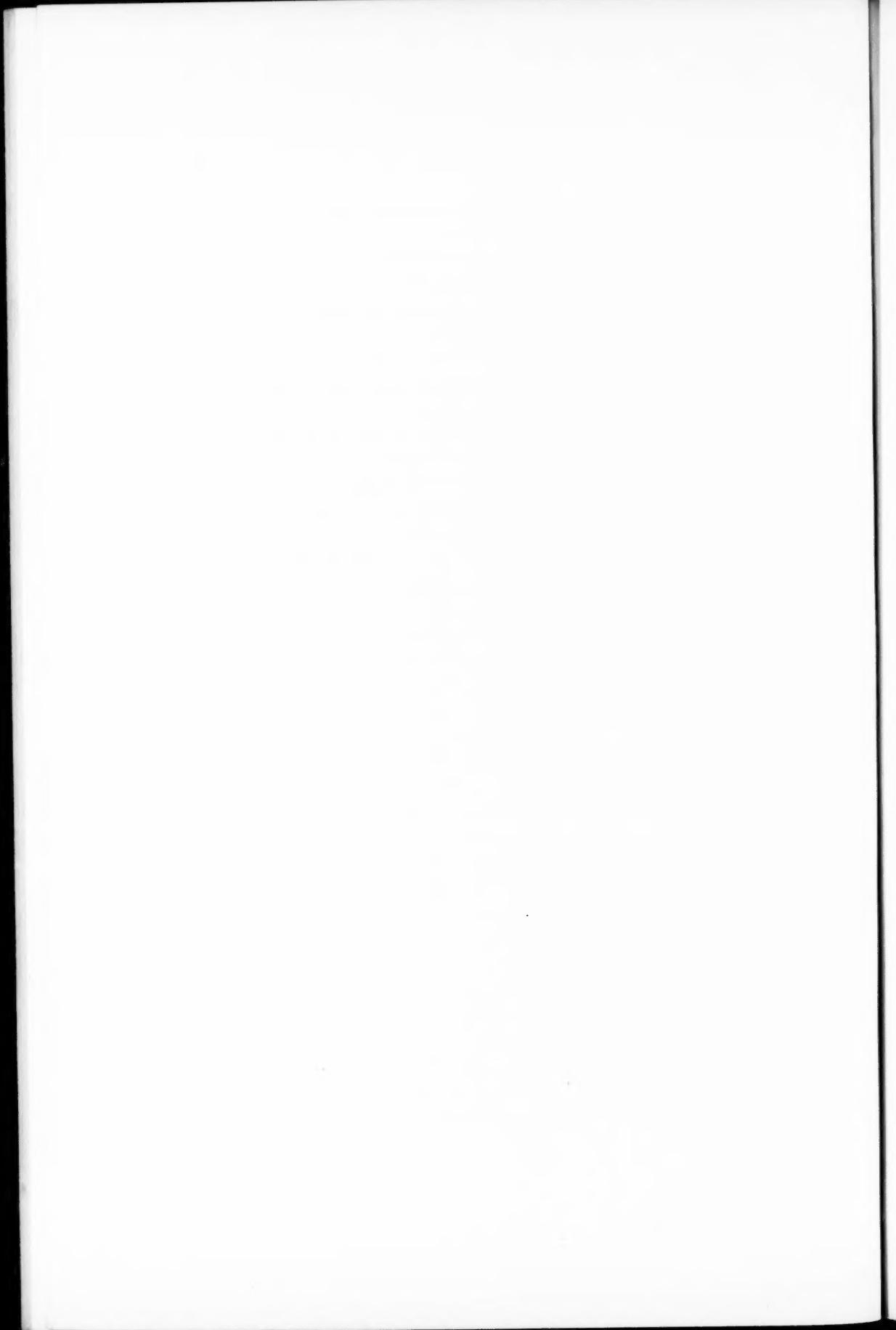
Whatever he does, or sees done, he should be primarily and acutely interested in its clinical significance, should endeavor to form his own estimate and should check this by observation of the subsequent course of events and by discussion or reading.

He should endeavor to make it a habit when collecting or examining a specimen, to ask himself:

1. What information will this examination furnish?
2. Would any other procedure be equally, or even more, useful?
3. What other information can the laboratory furnish in the study of this case?
4. Is this procedure related to: a) diagnosis; b) prognosis; or c) is it a means of suggesting appropriate methods of treatment or measuring their results?

The end and aim of the entire service, in short, should be to emphasize the fact that laboratory procedures are not merely "tests" but methods designed to ascertain and measure the response of the body to varied stimuli and to interpret these phenomena in terms of reaction to disease.

—R. A. KILDUFFE



NEWS AND NOTICES

THE TWELFTH ANNUAL CONVENTION

The Twelfth Annual Convention of the American Society of Clinical Pathologists will be held in Milwaukee June 9-12. A very elaborate program has been planned, however the local and program committee is not able at this time to give all of the details. An outstanding event will be a Symposium on the Medico-legal Autopsy which promises to contain one of the most useful groups of papers presented at our meetings.

The Program Committee would like to have authors send brief abstracts (not more than 200 words) of their papers at the earliest possible moment. These will be published in the May issue of the JOURNAL. This is purely voluntary on the part of the author and is being done as an experiment to see if it is worth while. Abstracts cannot be published unless they are in by April 10th.

The Scientific Committee would like to urge members to bring demonstrations of various tests, material and apparatus and to notify the Secretary as soon as possible concerning the amount of space desired.

The Research Committee would like to receive as much material as possible between now and the meeting in order that an adequate report can be made of it.

Clinical Pathologists and laboratory technicians will be interested in the following announcement:

Beginning April 1, 1933, applicants to the Registry must pass an examination conducted by a member of the American Society of Clinical Pathologists practicing in the locality in which the applicant resides. This examination will comprise:

- (a) An oral and practical test, counting fifty per cent,
- (b) Written test, twenty-five per cent,
- (c) Personal and psychological attributes, twenty-five per cent.

The fee for registration is Ten Dollars and is not returnable in case of failure. The applicant may, after the lapse of six months, be given the privilege of another examination without additional charge.

A notice has been received to the effect that the Journal of Preventive Medicine has been obliged, for financial reasons, to suspend publication at the end of the sixth volume. The American Journal of Hygiene will annually publish a number devoted to papers of the type which normally appeared in the Journal of Preventive Medicine. This number may be subscribed to separately.

The attention of clinical pathologists is invited once more to Biological Abstracts. The editors have succeeded in bringing out the Index to the second volume which is strongly recommended to members of the Society. This Index is the most comprehensive thing ever undertaken along this line for it is not only an index to abstracts but is a correlated summary of biologic sciences and shows clearly the inter-relation of different phases of biology. To the clinical pathologist this is a most valuable contribution and all members of the Society will profit by studying this Index volume very carefully. Other indices are in press and will be forthcoming shortly. Biological Abstracts needs support more than it ever did before. If this project of abstracting biological literature fails even the most optimistic doubt that it will ever be attempted again for years to come. The vast number of publications in the field, especially those dealing with medical subjects, is getting to be so large that the necessity of these abstracts becomes all too apparent to even the casual reader. Subscribe to Biological Abstracts and lend your support to this most worthy movement!

The LaMotte Chemical Products Company, regular advertisers in the JOURNAL, have specialized and originated the practical application of hydrogen ion concentration control. They have developed indicators, buffer salts and buffer mixtures as well as apparatus and sets for determining the hydrogen ion concentration of bacteriological culture media, sera, milk, urine and other fluids. Clinical pathologists can be assured of obtaining reliable reagents from this Company.

BOOK REVIEWS

Antony van Leeuwenhoek and His "Little Animals." By CLIFFORD DOBELL. Pp. 435. London, New York, Harcourt, Brace and Company, \$7.50.

Exactly 300 years ago there was born a simple unlettered Dutchman, who, at the age of forty-one, startled the Royal Society of London by announcing that he had discovered a new world. It was a world of little animals—"animalcules"—some so small that a thousand million of them were no larger than a grain of sand. He so convinced the ultrascientific Fellows of The Royal Society as to the truth and novelty of his observations that they made him one of their number. And in those days, as now, this was no small honor, because The Royal Society of that early date contained in its membership such intellectual giants as Robert Boyle, Robert Hooke, Christopher Wren and the omnipresent Samuel Pepys.

Clifford Dobell celebrates the tercentenary of the birth of this strange and simple Dutchman, Antony van Leeuwenhoek, by reintroducing him to us as the "Father of Bacteriology and Protozoology." Even though most Americans have avoided knowing much about Mr. van Leeuwenhoek, largely because they have been awed by the spelling of his name, and have despaired of ever correctly pronouncing it, Dobell, with great justice, urges us to meet his remarkable friend, shake his hand, and hearken to what he has to say to us, "for he is a man worth knowing more intimately. And though he was born exactly 300 years ago he is still very much alive and would be glad to make your better acquaintance—provided that 'you are a true lover of learning' (as of course you are)."

Of course, several hundred thousand Americans have already met Mr. van Leeuwenhoek, "The First of the Microbe Hunters," in Paul de Kruif's magnificent "Microbe Hunters." But the whole story of this splendid fellow could hardly be told in one

short chapter. Those whose appetites were whetted by de Kruif's story will find rich satisfaction in Dobell's biography, a labor of love which required twenty-five years of ardent research.

After one has jumped the hurdle of correctly pronouncing his name, which is much simpler than it looks (*Lay'-wen-hook*), one can settle back to several evenings of delightful entertainment with Dobell's book in his lap.

Because this great and simple man knew no language other than Nether-Dutch, and because he never wrote a book or a scientific paper—only letters and still more letters—his writings have suffered severe mutilations and perversions at the hands of his translators. After Dobell had discovered that Leeuwenhoek's original Dutch letters were, for the most part, extant in the Archives of The Royal Society in London, he abandoned all attempts to learn about the man from the writings of others.

To Dobell's delight he soon found that Leeuwenhoek knew no "science," for he was merely the proprietor of a dry-goods store and haberdashery, who also held the civic appointments of Wine Gauger and Chamberlain to the Sheriffs of his native town of Delft, which means, according to the terms of appointment that it was Leeuwenhoek's duty "to clean the aforesaid Chamber properly and to keep it neat and tidy." From his small income, this draper-haberdasher-wine gauger-janitor derived sufficient funds to permit him to conduct his explorations into a new and unsuspected world.

When not engaged in his janitorial duties, or selling buttons and ribbons, or testing wines, Leeuwenhoek ground and polished lenses and built his own "microscopes"—hundreds of them. They were simple affairs, consisting of a tiny matchhead size biconvex magnifying glass, mounted between silver, gold or brass plates. With these highly original instruments he discovered the weird and fantastic world of "little animals" in well-water, sea-water, snow-water, pepper-water, clove-water, nutmeg-water, or in the intestinal contents of horse flies, fleas, lice, maggots, snails, spiders, beetles, not to mention cows, calves, sheep, rabbits, whales, and human beings. He not only described with remarkable simplicity and accuracy free-living and parasitic protozoa

and bacteria, but he expressed a strong suspicion of the part played by putrefactive organisms in the general economy of nature—almost 200 years before Pasteur and Koch proved that these “little animals” were the chief murderers of mankind.

Doctor Thomas Molyneux, a contemporary Fellow of The Royal Society, after visiting Mr. van Leeuwenhoek described him as “A very civil compleasant man, & doubtless of great natural Abilities; but contrary to my Expectations quite a stranger to letters, . . . which is a great hindrance to him in his reasonings upon his Observations, for being ignorant of all other Mens thoughts, he is wholly trusting to his own.” Perhaps in this somewhat disparaging and patronizing description we have the very secret of Leeuwenhoek’s success, because as Leeuwenhoek himself said “novelties oft-times aren’t accepted, because men are apt to hold fast by what their Teachers have impressed on ‘em.”

Leeuwenhoek was thrilled by his own observations and he transmits his enthusiasm to the reader of his vivid descriptions. His discoveries are by no means limited to the field of bacteriology and protozoology, for his letters contain novel observations on matters zoological, botanical, chemical, physical, physiological and medical.

In his magnificently written and thoroughly documented biography, Dobell disperses the aura of fictional absurdities which has surrounded Leeuwenhoek’s accomplishments—and an even greater man emerges. In this respect, Dobell’s story of the life and accomplishments of Leeuwenhoek is the antithesis of the “debunking” trend of modern biographers.

The book is beautifully printed on fine stock, with a profusion of photolithographs and halftone plates. The education of every biologist is incomplete until he has absorbed its contents. But it is by no means a book for this small group of scientific workers alone. It is a story for all who wish to understand the true spirit of scientific inquiry, because the contents between its covers—set forth in a simple, beautiful and exciting manner—contain more of that spirit than any book I have ever read.

—WALTER M. SIMPSON

Streptococci in Relation to Man in Health and Disease. BY ANNA W. WILLIAMS. Pp. vi + 260, 1932, Baltimore, The Williams & Wilkins Company, \$5.00.

In this monograph the author, well known for her work on Streptococci, reviews the outstanding literature in the field. While her review is by no means as extensive as that published by the Pickett-Thompson Laboratory, it is far more readable and covers most of the important material published.

The book in part deals with the general characteristics of the group and their general manifestations and then specifically with local infections, elective localization, local immunity, erysipelas, scarlet fever, septic sore throat, the beta hemolytic group, the relation of streptococci to rheumatic fever, arthritis, measles, influenza, the common cold, poliomyelitis and encephalitis.

Much of the controversial part of the book centers around the work of Rosenow; evidently the author is not in agreement with him although she does not present sufficient evidence to match his thousands of experiments. These matters are forceably brought to the attention of the reader by such statements as "we all agree, except Rosenow and his associates" and, "the majority of us believe" which causes one to reflect on who "we all" really represent and how the "majority" was determined, if by actual count or by dead reckoning. The unbiased and critical reader will demand much more information than this book presents before supporting either side.

A table of 83 milk borne epidemics of scarlet fever and septic sore throat is especially valuable and well executed and the chapter on scarlet fever is very valuable and well done.

Two phases of the subject seem slighted: one, therapy in scarlet fever and what it has actually accomplished especially from a prophylactic standpoint, and second, the bacteriology of subacute bacterial endocarditis, which is covered in a scant page of text.

The book is certainly stimulating and although many will be disappointed in not finding more original work and more summaries and conclusions, great benefit will result from reading it.